

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|--|-----------|--|
| (51) International Patent Classification ⁵: C12N 15/13, C07K 15/28, A61K 39/395 | A1 | (11) International Publication Number: WO 94/25591 (43) International Publication Date: 10 November 1994 (10.11.94) |
| (21) International Application Number: PCT/EP94/01442 (22) International Filing Date: 28 April 1994 (28.04.94) (30) Priority Data: 93201239.6 29 April 1993 (29.04.93) EP (34) Countries for which the regional or international application was filed: NL et al. 93201454.1 19 May 1993 (19.05.93) EP (34) Countries for which the regional or international application was filed: NL et al. 93202079.5 15 July 1993 (15.07.93) EP (34) Countries for which the regional or international application was filed: NL et al. (71) Applicant (for all designated States except AU BB CA GB IE LK MN MW NZ SD US): UNILEVER N.V. [NL/NL]; Weena 455, NL-3013 AL Rotterdam (NL). (71) Applicant (for AU BB CA GB IE LK MN MW NZ SD only): UNILEVER PLC [GB/GB]; Unilever House, Blackfriars, London EC4 4BQ (GB). (71)(72) Applicants and Inventors: HAMERS, Raymond [BE/BE]; Vijversweg 15, B-1640 Sint-Genesius-Rode (BE). HAMERS-CASTERMAN, Cécile [BE/BE]; Vijversweg | | 15, B-1640 Sint-Genesius-Rode (BE). MUYLDERMANS, Serge, Victor, M. [BE/BE]; Brusselse Steenweg 55, B-1560 Hoeilaart (BE). (72) Inventors; and (75) Inventors/Applicants (for US only): FRENKEN, Leon, Gerardus, J. [NL/NL]; Geldersestraat 90, NL-3011 MP Rotterdam (NL). VERRIPS, Cornelis, Theodorus [NL/NL]; Hagedoorn 18, NL-3142 KB Maassluis (NL). (74) Common Representative: UNILEVER N.V.; Patent Division, P.O. Box 137, NL-3130 AC Vlaardingen (NL). (81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published With international search report. |
| (54) Title: PRODUCTION OF ANTIBODIES OR (FUNCTIONALIZED) FRAGMENTS THEREOF DERIVED FROM HEAVY CHAIN IMMUNOGLOBULINS OF CAMELIDAE | | |
| (57) Abstract <p>A process is provided for the production of an antibody or a fragment or functionalized fragment thereof using a transformed lower eukaryotic host containing an expressible DNA sequence encoding the antibody or (functionalized) fragment thereof, wherein the antibody or (functionalized) fragment thereof is derived from a heavy chain immunoglobulin of <i>Camelidae</i> and is devoid of light chains, and wherein the lower eukaryotic host is a mould, preferably belonging to the genera <i>Aspergillus</i> or <i>Trichoderma</i>, or a yeast, preferably belonging to the yeast genera <i>Saccharomyces</i>, <i>Kluyveromyces</i>, <i>Hansenula</i>, or <i>Pichia</i>. The heavy chain fragment can contain at least the whole variable domain. A complementary determining region (CDR) different from the CDR belonging to the natural antibody ex <i>Camelidae</i> can be grafted on the framework of the variable domain of the heavy chain immunoglobulin. The catalytic antibodies can be raised in <i>Camelidae</i> against transition state molecules. The functionalized antibody or fragment thereof can comprise a fusion protein of both a heavy chain immunoglobulin from <i>Camelidae</i> or a fragment thereof and another polypeptide, e.g., an enzyme, preferably an oxido-reductase. Also provided are new products obtainable by a process as described, and compositions containing a product produced by a process as described, which composition may contain a new product as provided.</p> | | |

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|----|--------------------------|----|---------------------------------------|----|--------------------------|
| AT | Austria | GB | United Kingdom | MR | Mauritania |
| AU | Australia | GE | Georgia | MW | Malawi |
| BB | Barbados | GN | Guinea | NE | Niger |
| BE | Belgium | GR | Greece | NL | Netherlands |
| BF | Burkina Faso | HU | Hungary | NO | Norway |
| BG | Bulgaria | IE | Ireland | NZ | New Zealand |
| BJ | Benin | IT | Italy | PL | Poland |
| BR | Brazil | JP | Japan | PT | Portugal |
| BY | Belarus | KE | Kenya | RO | Romania |
| CA | Canada | KG | Kyrgyzstan | RU | Russian Federation |
| CF | Central African Republic | KP | Democratic People's Republic of Korea | SD | Sudan |
| CG | Congo | KR | Republic of Korea | SE | Sweden |
| CH | Switzerland | KZ | Kazakhstan | SI | Slovenia |
| CI | Côte d'Ivoire | LI | Liechtenstein | SK | Slovakia |
| CM | Cameroon | LK | Sri Lanka | SN | Senegal |
| CN | China | LU | Luxembourg | TD | Chad |
| CS | Czechoslovakia | LV | Latvia | TG | Togo |
| CZ | Czech Republic | MC | Monaco | TJ | Tajikistan |
| DE | Germany | MD | Republic of Moldova | TT | Trinidad and Tobago |
| DK | Denmark | MG | Madagascar | UA | Ukraine |
| ES | Spain | ML | Mali | US | United States of America |
| FI | Finland | MN | Mongolia | UZ | Uzbekistan |
| FR | France | | | VN | Viet Nam |
| GA | Gabon | | | | |

Title: **Production of antibodies or (functionalized) fragments thereof derived from heavy chain immunoglobulins of *Camelidae***

The present invention relates to a process for the production of antibodies or
5 (functionalized) fragments thereof derived from heavy chain immunoglobulins of *Camelidae* and is partly based on research investigations carried out at the Free University of Brussels. A draft publication thereon already submitted to the periodical Nature and communicated to the present applicants by Prof. R. Hamers reads as follows.

10

FUNCTIONAL HEAVY CHAIN IMMUNOGLOBULINS IN THE CAMELIDS

Random association of V_L and V_H repertoires contributes considerably to antibody diversity (1). The diversity and the affinity are then increased by hypermutation in
15 B-cells located in germinal centres (2). Except in the heavy chain disease (3), naturally occurring heavy chain antibodies have not been described, although antigen binding has been demonstrated for separated heavy chains (4) or cloned V_H domains (5). The presence of considerable amounts IgG like material of 100 Kd in the serum of the camel (*Camelus dromedarius*) (6) was confirmed. These molecules
20 are composed of heavy chain dimers and are devoid of light chains. Nevertheless they bear an extensive antigen binding repertoire, a finding which questions the role of the light chains in the camel. Camel heavy chain IgGs lack the C_H1 , which in one IgG class might be structurally replaced by an extended hinge. Heavy chain IgGs are a feature of all camelids. These findings open perspectives in engineering of
25 antibodies.

By a combination of affinity chromatography on Protein A and Protein G, three quantitatively important fractions corresponding to subclasses of IgG can be isolated from the serum of camels (*Camelus dromedarius*) (Fig. 1A, lanes c-f).
30 One fraction (IgG_1) contains molecules of 170 Kd (Fig. 1B, lane 2) which upon reduction yield 50 Kd heavy chains and large 30 kD light chains (Fig. 1C, lane 2). The two other immunoglobulin fractions contain molecules of approximately 100 Kd

(Fig. 1B, lanes 1 and 3) which upon reduction yield only heavy chains of respectively 46 Kd (IgG₂ fraction binding only to Protein A) (Fig. 1C, lane 3) and 43 Kd (IgG₃ fraction binding to Protein A and Protein G) (Fig. 1C, lane 1). These two IgG classes appear to lack the light chain completely.

5

To exclude the possibility that the light chains were only weakly associated with the heavy chains and lost during the selective purification, whole serum was size fractionated by gel filtration. Coomassie blue staining of unreduced fractions revealed the sequential elution of the 170 Kd IgG₁ followed by the incompletely resolved isotypes IgG₂ and IgG₃ (90 Kd) (Fig. 1D, upper inset). Immunostaining of the same fractions after reduction confirmed that the light chains were present solely in the 50 Kd heavy chain containing fractions (Fig. 1D, lower inset).

A comparative study of old world camelids (*Camelus bactrianus* and *Camelus dromedarius*) and new world camelids (*Lama pacos*, *Lama glama* and *Lama vicugna*) showed that heavy chain immunoglobulins are abundant in the sera of all species examined (data not shown) and total up to 75% of the molecules binding to protein A.

The abundance of the heavy chain immunoglobulins in the serum of camelids raises the question as to whether they bear an extensive antigen binding repertoire. This question could be answered by examining the IgG₁, IgG₂ and IgG₃ fractions from the serum of camels (*Camelus dromedarius*) with a high antitrypanosome titer (7). In radio-immunoprecipitation, purified fractions of IgG₁, IgG₂ and IgG₃ derived from infected camels were shown to bind a large number of antigens present in a ³⁵S methionine labelled trypanosome lysate (Fig. 2A), indicating an extensive repertoire complexity for the three IgG classes. Conversely, in blotting experiments, ³⁵S methionine labelled trypanosome lysate binds to SDS-PAGE separated IgG₁, IgG₂ and IgG₃ obtained from infected animals (Fig. 2B). These findings indicate that the heavy chains alone can generate an extensive repertoire and question the obligatory contribution of the light chain to the useful antibody repertoire in the camelids.

The camelid $\gamma 2$ and $\gamma 3$ chains are considerably shorter than the normal mammalian γ or camel $\gamma 1$ chains. This would suggest that, as in the case of heavy chain disease (3), deletions have occurred in the C_{H1} protein domain (8,9). To address this question, cDNA was synthesized from camel spleen mRNA and the sequences
5 between the 5' end of the V_H and the C_{H2} were amplified by a Polymerase Chain Reaction (PCR), and cloned. Seventeen clones presenting a different V_H sequence were isolated and sequenced. Their most striking feature was the complete lack of the C_{H1} domain, the last framework (FR4) residues of the V_H region being immediately followed by the hinge (Fig. 3, lower part). The absence of the C_{H1} domain
10 clarifies two important dilemmas.

First, immunoglobulin heavy chains are normally not secreted unless the heavy chain chaperoning protein or BIP (10) has been replaced by the L chain (11), or alternatively the C_{H1} domain has been deleted (3,8,9). Secondly, isolated heavy
15 chains from mammalian immunoglobulins tend to aggregate, but are only solubilized by light chains (8,12) which bind to the C_{H1} and the V_H domains (13).

14 of the 17 clones were characterized by a short hinge sequence with a length equal to that of human IgG₂ and IgG₄ (14) (Fig. 3). The other 3 had a long hinge
20 sequence containing the 'EPK' hinge motif found in human IgG₁ and IgG₃ (14). They possess the C_{H2} 'APELL/P' motif also found in human IgG₁ and IgG₃ (see SEQ. ID. NO: 1-2), and which is associated with mammary transport of bovine IgG₁ (15). On basis of molecular weight, we expect the "short hinge" clones to correspond to IgG₃ and the "long hinge" clones to IgG₂.

25 In the short hinge containing antibody, the extreme distance between the extremities of the V_H regions will be of the order of 80 Å corresponding to twice the size of a single domain of 40 Å ($2 \times V_H$) (16). This could be a severe limitation for agglutinating, cross linking or complement fixation (17,18). In the long hinge containing
30 immunoglobulin the absence of C_{H1} might be compensated by the extremely long hinge itself, composed of a 12 fold repeat of the sequence Pro-X (X=Gln, Glu, Lys) (Fig. 3 & 4). NMR (19) and molecular modelling (20) of Pro-X repeats present in

the TonB protein of *E. coli* (X=Glu, Lys) and the membrane procyclin of trypanosomes (X=Asp, Glu) indicate that these repeated sequences function as rigid rodlike spacers with a diameter of 8 Å and a rise of 2.9 Å per residue. Assuming the same geometry, the long hinge would be 70 Å which compensates for the absence of the C_H1 domain.

The binding site of heavy chain antibodies cannot form the pocket resulting from adjoining light and heavy chain V regions and the residues of the V_H which normally interact with V_L will be exposed to solvent (3,5,13). It was found that leucine at position 45 conserved in 98% of human and murine V_H sequences (14), and crucial in the V_H-V_L association (13), can be replaced by an arginine (Fig. 3, upper part). This substitution is in accordance with both the lost contact with a V_L domain and an increased solubility.

Unlike myeloma heavy chains which result mainly from C_H1 deletion in a single antibody producing cell (21) the camelid heavy chain antibodies have emerged in a normal immunological environment and it is expected that they will have undergone the selective refinement in specificity and affinity accompanying B cell maturation (1, 2). The obtention of camelid heavy chain antibodies could therefore be an invaluable asset in the development and engineering of soluble V_H domains (5) or of new immunologicals for diagnostic, therapeutic or biochemical purposes.

REFERENCES

1. Tonegawa, S. *Nature* **302**, 575-581 (1983).
- 25 2. Jacob, J., Kelsoe, G., Rajewski, K., & Weiss, U. *Nature* **354**, 389-392 (1991).
3. Fleischman J.B., Pain R.H. & Porter R.R. *Arch.Biochem.Biophys* **Suppl. 1**, 174-180 (1962).
4. Utsumi, S. & Karush, F. *Biochemistry* **3**, 1329-1338 (1964).
5. Ward, E.S., Güssow, D., Griffiths, A.d., Jones, P.T. & Winter G. *Nature* **341**, 544-546 (1989).
- 30 6. Ungar-Waron H., Eliase E., Gluckman A. and Trainin Z. *Isr. J. Vet. Med.* **43**, 198-203 (1987).

7. Bajjana Songa, E., & Hamers R. *Ann.Soc.Belge Méd.Trop.* 68, 233-240 (1988).
8. Seligmann M., Mihaesco E., Preud'homme J.-L., Danon F. & Brouet J.-C. *Immun.Rev.* 48, 145-167 (1979).
9. Traunecker, A., Schneider, J., Kiefer, H., Karjalainen, K., *Nature* 339, 68-70
5 (1989).
10. Hendershot L.M., Bole D., Köhler, G. & Kearney, J.F. *J. Cell Biol.* 104, 761-767 (1987).
11. Hendershot L.M. *J.Cell Biol.* 111, 829-837 (1990).
12. Røhlt O., Onoue K. & Pressman D. *Proc.Natn.Acad.Sci. USA* 51, 173-178
10 (1964).
13. Chothia, C., Novotny, J., Bruccoleri, R., Karplus, M. *J. Mol.Biol.* 186, 651-663 (1985).
14. Kabat E.A., Wu, T.T., Reid-Miller, M., Perry H.M. & Gottesman, K.S. *Sequences of Proteins of Immunological Interest* 511 (U.S. Dept of Health and Human Services,
15 US Public Health Service, National Institutes of Health, Bethesda, 1987).
15. Jackson, T., Morris, B.A., Sanders, P.G. *Molec.Immun.* 29, 667-676 (1992).
16. Poljak R.J. *et al. Proc.Natn.Acad.Sci. USA* 70, 3305-3310 (1973).
17. Dangl J.L., *et al. EMBO J.* 7, 1989-1994 (1988).
18. Schneider W.P. *et al. Proc.Natn.Acad.Sci USA* 85, 2509-2513 (1988).
- 20 19. Evans, J.S. *et al. FEBS Lett.* 208, 211-216 (1986).
20. Roditi, I. *et al. J.Cell Biol.* 108, 737-746 (1989).
21. Dunnick, W., Rabbits, T.H., Milstein, C. *Nucl.Acids Res.*, 8, 1475-1484 (1980).
22. Bülow, R., Nonnengässer, C., Overath, P. *Mol.Biochem.Parasitol.* 32, 85-92 (1989).
- 25 23. Sambrook, J., Fritsch, E.F. & Maniatis, T. *Molecular Cloning: A Laboratory Manual* 2nd Edn (Cold Spring Harbor Laboratory Press, New York, 1989).
24. Sastry, L *et al. Proc.Natn.Acad.Sci. USA* 86, 5728-5732 (1989).
25. Sanger, F., Nicklen, S. & Coulson, A.R. *Proc.Natn.Acad.Sci. USA* 74, 5463-5467 (1977).
- 30 26. Klein, J. *Immunology* (Blackwell Scientific Publications, London, 1990).

Figure 1 Characterisation and purification of camel IgG classes on Protein A, Protein G and gel filtration.

- (A) The fraction of *C. dromedarius* serum adsorbed on Protein A shows upon reduction on SDS-PAGE three heavy chain components of respectively 50, 46, and 43 Kd (bands between dots), absent in the non adsorbed fraction (lane d), and light chain components of around 30 Kd (lane c) considerably larger than rabbit light chain (lane a, rabbit IgG). The fractions adsorbed on Protein G (lane e) lack the 46 Kd heavy chain which remains in the non adsorbed fraction (lane f). Lane b contains a size marker.
- 10 (B and C) By differential adsorption and elution on Protein G and Protein A, the IgG fractions containing 43 Kd (lane 1), 46 Kd (lane 3) and 50 Kd (lanes 2) heavy chains were purified and analysed on SDS-PAGE in absence (B) or presence (C) of DTT.
- (D) Whole camel serum (0.1 ml) was fractionated by gel filtration on a
15 Superdex 200 column using 150 mM NaCl, 50 mM sodium phosphate buffer pH 7.0 as eluent. Affinity purified IgG₂ and IgG₃ elute at the positions indicated by arrows. The fractions of interest were further analysed by SDS-PAGE with or without prior reduction. The protein contents as visualized by Coomassie blue (without reduction, upper inset) are compared with the immunoglobulins from the same fractions (after
20 reduction with DTT, lower inset) as revealed by Western blotting with a rabbit anti-camel-IgG (lower inset).
- METHODS.** 5 ml of *C. dromedarius* serum is adsorbed onto a 5 ml Protein G Sepharose (Pharmacia) column, and washed with 20 mM phosphate buffer, pH 7.0.
25 Upon elution with 0.15 M NaCl, 0.58 % acetic acid (pH 3.5), IgG₃ of 100 Kd is eluted which upon reduction yields heavy chains of 43 Kd (lane 1, B and C). IgG₁ of 170 Kd can subsequently be eluted with pH 2.7 buffer (0.1 M Gly-HCl). This fraction, upon reduction, yields a 50 Kd heavy chain and a broad light chain band (lane 2, C). The fraction not adsorbed on Protein G is brought on a 5 ml Protein A
30 Sepharose column. After washing and elution with 0.15 M NaCl, 0.58% acetic acid (pH 4.5) IgG₂ of 100 Kd is obtained which consists solely of 46 Kd heavy chains (lane 3, C).

Figure 2 Repertoire complexity and antigen binding capacity of camel IgG₁, IgG₂ and IgG₃ analysed by radioimmunoprecipitation (A) or Western blotting (B & C).

- (A) Serum or purified IgG fractions from healthy or *Trypanoma evansi* infected *C. dromedarius* (CATT titer 1/160 (7)) were incubated with labelled trypanosome lysate, recovered with Protein A Sepharose and analysed by SDS-PAGE. The relative counts recovered are inscribed below each lane. No trypanosome proteins bind to the Protein A or to the healthy camel immunoglobulins.
- 10 (B) 20 µg of IgG₁, IgG₂ and IgG₃ from healthy and trypanosome infected animals were separated by SDS-PAGE without prior reduction or heating. The electroblotted proteins were incubated with the labelled trypanosome lysate. The IgG₂ shows a single antigen binding component corresponding to the heavy chain immunoglobulin whereas the IgG₃ fraction appears to contain in addition two
- 15 larger antigen binding components barely detectable by Ponceau Red staining (C). These are possibly Ig classes copurified as immunocomplexes present in the serum of the infected animals.

- METHODS.** (³⁵S)-methionine labelled *Trypanosoma evansi* lysate (500,000 counts)
- 20 (22) was incubated (4°C, 1 hour) with 10 µl of serum or, 20 µg of IgG₁, IgG₂ or IgG₃ in 200 µl of 0.4 M NaCl, 10 mM EDTA, 10 mM Tris (pH 8.3), containing 0.1 M TLCK. 10 mg of Protein A Sepharose suspended in 200 µl of the same buffer was added (4°C, 1 hour). After washing and centrifugation, each pellet was resuspended
- 25 in 75 µl SDS PAGE sample solution containing DTT, and heated for 3 min. at 100°C. After centrifugation, 5 µl of the supernatant was saved for radioactivity counting and the remainder analysed by SDS PAGE and fluorography.
- The nitrocellulose filter of the Western blot of purified fractions IgG₁, IgG₂ and IgG₃ was stained with Ponceau Red (C) or incubated with 1% ovalbumin in TST buffer (Tris 10 mM, NaCl 150 mM, Tween 0,05%) (B). The membrane was
- 30 extensively washed with TST buffer and incubated for 2 hours with (³⁵S)-labelled trypanosome antigen. To avoid unspecific binding, the labelled trypanosome antigen

lysate was filtered (45 μ) and incubated with healthy camel immunoglobulin and ovalbumin adsorbed on a nitrocellulose membrane.

Figure 3 Amino acid sequences of the V_H framework, and hinge/C_H2 of *Camelus dromedarius* heavy chain immunoglobulins, compared to human (italic) V_H framework (subgroup III) and hinges of human IgG (14).

METHODS. Total RNA was isolated from a dromedary spleen (23). mRNA was purified with oligo T-paramagnetic beads (PolyAtract-Promega). 1 μ g mRNA was used for preparing double-strand cDNA (23) after an oligo-dT priming using enzymes provided by Boehringer Mannheim. 5 μ g of cDNA was amplified by PCR in a 100 μ l reaction mixture (10mM Tris-HCl pH 8.3, 50 mM KCl, 15 mM MgCl₂, 0.01% (w/v) gelatine, 200 μ M of each dNTP). 25 pmoles of each oligonucleotide of the mouse V_H (24), containing a XhoI site, and 5'-CGCCATCAAGGTACCAGT-TGA-3' (see SEQ. ID. NO: 3) were used as primers. The 3' end primer was deduced from partial sequences corresponding to γ chain amino acid 296 to 288 (T. Atarhouch, C. Hamers-Casterman, G. Robinson, private communication) in which one mismatch was introduced to create a KpnI restriction site. After a round of denaturing annealing (94°C for 5 min. and 54°C for 5 min.), 2 U of Taq DNA polymerase were added, to the reaction mixture before subjecting it to 35 cycles of amplification (5). The PCR products were purified by phenol-chloroform extraction followed by HPLC (Genpak-fax column, Waters) and finally by MERMAID (BIO 101, Inc.). After these purification steps, the amplified cDNA was digested with XhoI and KpnI, and ligated into pBluescript. The clones were sequenced by the dideoxy chain termination method (25). The sequences were translated into amino acids which allowed their assignment to well defined domains of the Ig molecule (14); see SEQ. ID. NO: 4-12

Figure 4 Schematic representation of the structural organisation of the camel immunoglobulins (adapted from 26).

On the basis of size consideration, the IgG₁ fraction possess probably the normal antibody assembly of two light and two heavy chains. IgG₃ would have a hinge comparable in size to the human IgG₁, IgG₂ and IgG₄. The two antigen binding sites

are much closer to each other as this camel IgG lacks the C_H1 domain. In the camel IgG₂ the long hinge, being formed of Pro-X repeats (X = Glu, Gln or Lys), most likely adopt a rigid structure (19,20). This long hinge could therefore substitute the C_H1 domain and bring the two antigen binding sites of IgG₂ to normal positions.

5

--- End of Draft publication ---

Background of the invention

Already at a very early stage during evolution antibodies have been developed to
10 protect the host organisms against invading molecules or organisms. Most likely one of the earliest forms of antibodies must have been developed in *Agnatha*. In these primitive fishes antibodies of the IgM type consisting of heavy and light chains have been detected. Also in many other forms of life ranging from amphibians to
15 mammals antibodies are characterized by the feature that they consist of two heavy and two light chains, although the heavy chains of the various classes of immunoglobulins are quite different. These heavy and light chains interact with each other by a number of different physical forces, but interactions between hydrophobic patches present on both the heavy and light chain are always important. The interaction between heavy and light chains exposes the complementarity determining
20 regions (CDRs) of both chains in such a way that the immunoglobulin can bind the antigen optimally. Although individual heavy or light chains have also the capability to bind antigens (Ward *et al.*, Nature 341 (1989) 544-546 = ref. 5 of the above given draft publication) this binding is in general much less strong than that of combined heavy and light chains.
25 Heavy and light chains are composed of constant and variable domains. In the organisms producing immunoglobulins in their natural state the constant domains are very important for a number of functions, but for many applications of antibodies in industrial processes and products their variable domains are sufficient. Consequently many methods have been described to produce antibody fragments.

30

One of these methods is characterized by cleavage of the antibodies with proteolytic enzymes like papain and pepsin resulting in (a) antibody fragment comprising a light

chain bound via an S-S bridge to part of a corresponding heavy chain formed by proteolytic cleavage of the heavy chain (Fab), or (b) a larger fragment of the antibody comprising two of these Fabs still connected to each other via an S-S bridge in enlargements of the heavy chain parts, indicated with $F(ab)_2$, respectively
5 (see patent applications EP-A-0125023 (GENENTECH / Cabilly *et al.*, 1984) and WO-A-93/02198 (TECH. RES. CENT. FINLAND / Teeri *et al.*, 1993) for definitions of these abbreviations). The disadvantage of the enzymatic route is that the production of whole antibodies is expensive and the enzymatic processing increases the costs of these fragments even more. The high costs of antibody fragments block
10 the application of these fragments in processes and products outside the pharmaceutical industry.

Another method is based on linkage on DNA level of the genes encoding (parts of) the heavy chain and the light chain. This linkage and the subsequent production of
15 these chimeric immunoglobulins in microorganisms have been described (for Fab fragments see e.g. Better *et al.*, Science 240 (1988) 1041-1043, for F_v fragments (combination of variable fragments of the heavy chain (V_H) and light chain (V_L) still connected to each other by non-covalent binding interactions) see e.g. Skerra *et al.*, Science 240 (1988) 1938, and for single chain F_v fragments (ScF_v ; an F_v fragment in
20 which the two variable fragments are linked to each other by a linker peptide) see e.g. Bird *et al.*, Science 242 (1988) 423-426. Provided that an appropriate signal sequence has been placed in front of the single chain V_H and V_L antibody fragment (ScF_v), these products are translocated in *E. coli* into the periplasmic space and can be isolated and activated using quite elaborate and costly procedures. Moreover the
25 application of antibody fragments produced by *E. coli* in consumer products requires extensive purification processes to remove pyrogenic factors originating from *E. coli*. For this and other reasons the production of ScF_v in microorganisms that are normally used in the fermentation industry, like prokaryotes as *Streptomyces* or *Bacillus* (see e.g. Wu *et al.* Bio/Technology 11 (1993) 71) or yeasts belonging to the
30 genera *Saccharomyces* (Teeri *et al.*, 1993, *supra*), *Kluyveromyces*, *Hansenula*, or *Pichia* or moulds belonging to the genera *Aspergillus* or *Trichoderma* is preferred. However with a very few exceptions the production of ScF_v antibodies using these systems

proved to be impossible or quite poor. Although the exact reasons for the poor production are not well known, the use of linkers between the V_H and V_L chains not designed for secretion (Teeri *et al.*, 1993, *supra*) may be a reason.

- 5 Another reason may be incorrect folding of ScF_v . The frameworks and to a limited extend the CDRs of variable domains of light and heavy chains interact with each other. It has been described by Chothia *et al.* (J. Mol. Biol. 186 (1985) 651-663 = ref. 13 of the above given draft publication) that this interaction involves amino acids at the following positions of the variable region of the heavy chain: 35, 37, 39,
10 44-45, 47, 100-103 and 105 (numbering according to Kabat *et al.*, In "*Sequences of Proteins of Immunological Interest*", Public Health Service, NIH, Washington DC, 1983 = ref. 14 of the above given draft publication). Especially leucine at position 45 is strongly conserved and the whole apolar side chain of this amino acid seems to be involved in the interaction with the light chain. These strong interactions may
15 fold the ScF_v into a structure that can not be translocated in certain types of lower eukaryotes.

Thus the use of a linker in the production of ScF_v for connecting a V_H chain to a V_L chain, might negatively influence either the translocation, or the folding of such ScF_v
20 or both.

Not prior-published European patent application 92402326.0 filed 21.08.92 (C. Casterman & R. Hamers) discloses the isolation of new animal-derived immunoglobulins devoid of light chains (also indicated as heavy chain immunoglobulins),
25 which can especially originate from animals of the camelid family (*Camelidae*). This European patent specification, now publicly available as EP-A1-0 584 421, is incorporated herein by reference. These heavy chain immunoglobulins are characterized in that they comprise two heavy polypeptide chains sufficient for the formation of one or more complete antigen binding sites, whereby a complete antigen binding
30 site means a site which will alone allow the recognition and complete binding of an antigen, which can be verified by any known method regarding the testing of the binding affinity. The European patent specification further discloses methods for

isolating these heavy chain immunoglobulins from the serum of *Camelidae* and details of the chemical structure of these heavy chain immunoglobulins. It also indicates that these heavy chain immunoglobulins and derivatives thereof can be made by using recombinant DNA technology in both prokaryotes and eukaryotes. The
5 present invention relates to a further development of the work disclosed in that prior-filed but not prior-published European specification.

Due to the absence of light chains in most of the immunoglobulins of *Camelidae* such linkers are not necessary, thereby avoiding the above-mentioned potential
10 problems.

As described above in the draft publication for Nature, now publicly available as Nature 363 (3 June 1993) 446-448, and in the not prior-published European patent application 92402326.0 (*supra*) it was surprisingly found that the majority of the protein A-binding immunoglobulins of *Camelidae* consists just of two heavy chains
15 and that these heavy chains are quite different from common forms of heavy chains, as the C_H1 domain is replaced by a long or short hinge (indicated for IgG₂ and IgG₃, respectively, in Figure 4 of the above given draft publication for Nature).

Moreover these heavy chains have a number of other features that make them remarkably different from the heavy chains of common immunoglobulins.
20 One of the most significant features is that they contain quite different amino acid residues at those positions involved in binding to the light chain, which amino acids are highly conserved in common immunoglobulins consisting of two heavy and two light chains (see Table 1 and SEQ. ID. NO: 13-31).

Table 1 Comparison of amino acid sequences of various immunoglobulins

Alignment of a number of V_H regions of Camel heavy chain antibodies compared with those of mouse (M, top line) and human (H, second line). Framework fragments are indicated in capitals, CDR fragments in small print; see SEQ. ID.

- 5 NO: 13-31 for sequences indicated by M, H, 1, 2, 3, 7, 9, 11, 13, 16, 17, 18, 19, 20, 21, 24, 25, 27, 29, respectively.

| | | | | | | |
|----|-------|------------|-------------|------------|-------------|------------|
| | | 1 | | | | 50 |
| 10 | m | EVKLVESGGG | LVQPGGSLRL | SCATSGFTFS | dfyme..WVR | QP̄GKRLEWI |
| | h | EVQLVESGGG | LVQPGGSLRL | SCAASGFTFS | syams..WVR | QAPGKGLEWV |
| | cam1 |GG | SVQAGGSLRL | SCAASGYSNC | pltws..WYR | QFPGTEREFV |
| | cam2 | DVQLVASGGG | SVQAGGSLRL | SCTASGDSFS | rfams..WFR | QAPGKECELV |
| | cam3 |GG | SVQTGGSLRL | SCAVSGFSFS | tscma..WFR | QASGKQREGV |
| 15 | cam7 |GG | SVQGGGSLRL | SCAISGYTYG | sfcmg..WFR | EGPGKEREGI |
| | cam9 |GG | SVQAGGSLTL | SCVYTNDTGT | ...mg..WFR | QAPGKECERV |
| | cam11 |GG | SVQAGGSLRL | SCNVSGSPSS | tyclg..WFR | QAPGREREGV |
| | cam13 |GG | SVEAGGSLRL | SCTASGYVSS | ...ma..WFR | QVPGQEREGV |
| | cam16 |GG | SAQAGGSLRL | SCAAHGIPLN | gyyia..WFR | QAPGKGREGV |
| 20 | cam17 |GG | SVQPGGSLTL | SCTVSGATYS | dysig..WIR | QAPGKDREVV |
| | cam18 |GG | SVQAGGSLRL | SCTGSGFPYS | tfclg..WFR | QAPGKEREGV |
| | cam19 |GG | SVQAGGSLRL | SCAASDYTIT | dycma..WFR | QAPGKERELV |
| | cam20 |GG | SVQVGGSLRL | SCVASTHTDS | stcig..WFR | QAPGKEREGV |
| | cam21 |GG | SVQVGGSLKL | SCKISGGTPD | rvpkslawFR | QAPEKEREGI |
| 25 | cam24 |GG | SVQAGGSLRL | SCNVSGSPSS | tyclg..WFR | QAPGKEREGV |
| | cam25 |GG | SVQTGGSLRL | SCEISGLTFD | dsdvq..WYR | QAPGDECKLV |
| | cam27 |GG | SVQAGGSLRL | SCASSSKYMP | ctydmr..WYR | QAPGKEREFV |
| | cam29 |exxGG | SVQAGGSLRL | SCVASGFNFE | tsrma..WYR | QTPGNVCELV |
| 30 | | | | | | |
| | | 51 | | | | 100 |
| | m | A..asrnkan | dytteysasv | kgRFIVSRDT | SQSILYLQMN | ALRAEDTAIY |
| | h | S..xisxkt | ggxtyyadsv | kgRFTISRDN | SKNTLYLQMN | SLRAEDTAVY |
| | cam1 | S..smd...p | dgntkytysv | kgRFTMSRGS | TEYTVFLQMD | NLKPEDTAMY |
| 35 | cam2 | S..siq...s | ngrtteadsv | qgRFTISRDN | SRNTVYLQMN | SLKPEDTAVY |
| | cam3 | Aainsgggrt | yyntyvaesv | kgRFAISQDN | AKTTVYLDMN | NLTPEDTATY |
| | cam7 | A..tiln..g | gtntytyadsv | kgRFTISQDS | TLKTMVLLMN | NLKPEDTGTY |
| | cam9 | A..hit...p | dgmtfidepv | kgRFTISRDN | AQKTLSLRMN | SLRPEDTAVY |
| | cam11 | T..aint..d | gsiiyaadsv | kgRFTISQDT | AKETVHLQMN | NLQPEDTATY |
| 40 | cam13 | A..fvqt..a | dnsalygdsv | kgRFTISHDN | AKNTLYLQMR | NLQPDGTGVY |
| | cam16 | A..ting..g | rdvtyyadsv | tgRFTISRDS | PKNTVYLQMN | SLKPEDTAIY |
| | cam17 | A..aant..g | atskfyvdfv | kgRFTISQDN | AKNTVYLQMS | FLKPEDTAIY |
| | cam18 | A..gins..a | ggntytyadav | kgRFTISQGN | AKNTVFLQMD | NLKPEDTAIY |
| | cam19 | A.aiqvvrds | trltdyadsv | kgRFTISQGN | TKNTVNLQMN | SLTPEDTAIY |
| 45 | cam20 | A..siyf..g | dggtnyrds | kgRFTISQLN | AQNTVYLQMN | SLKPEDSAMY |
| | cam21 | A..vlst..k | dgktfyadsv | kgRFTIFLDN | DKTTFSLQLD | RLNPEDTADY |
| | cam24 | T..aint..d | gsviyaadsv | kgRFTISQDT | AKKTVYLQMN | NLQPEDTATY |
| | cam25 | Sgilsdgtpy | tksgdyaesv | rgRVTISRDN | AKNMIYLQMN | DLKPEDTAMY |
| | cam27 | S..sin...i | dgktttyadsv | kgRFTISQDS | AKNTVYLQMN | SLKPEDTAMY |
| 50 | cam29 | S..siy...s | dgktyyvdrn | kgRFTISRDN | AKNTLYLQLS | GLKPEDTAMY |

Table 1 (Cont.) Comparison of amino acid sequences of various immunoglobulins
Alignment of a number of V_H regions of Camel heavy chain antibodies compared
with those of mouse (M, top line) and human (H, second line). Framework
fragments are indicated in capitals, CDR fragments in small print; see SEQ. ID.

5 NO: 13-31 for sequences indicated by M, H, 1, 2, 3, 7, 9, 11, 13, 16, 17, 18, 19, 20,
21, 24, 25, 27, 29, respectively.

| | | | | |
|----|-------|-------------|-----------------------|-----------|
| | | 101 | | 139 |
| 10 | m | YCARdyygss |y.. f...dvWG | AGTTVTVSS |
| | h | YCARxxxxxx | xxxxxyyyh x....fdyWG | QGTLVTVSS |
| | cam1 | YCKTalqpgg | ycgygx....clWG | QGTQVTVSS |
| | cam2 | YCGAvslmdr | isqh.....gcRG | QGTQVTVSL |
| | cam3 | YCAAvpahlg | pgaildlkkykyWG | QGTQVTVSS |
| 15 | cam7 | YCAAelsggs | celpllf...dyWG | QGTQVTVSS |
| | cam9 | YCAAdwkywt | cgaqtggyf.gqWG | QGAQVTVSS |
| | cam11 | YCAArItemg | acdarwatla trtfaynyWG | QGTQVTVSS |
| | cam13 | YCAAgkkdrt | rwaeprew..nnWG | QGTQVTASS |
| | cam16 | FCAAgstrfss | pvgstsrles .sdy..nyWG | QGIQVTASS |
| 20 | cam17 | YCAAadpsiy | ysilxiey..kyWG | QGTQVTVSS |
| | cam18 | YCAAdspcym | ptmpappird sfgw..ddFG | QGTQVTVSS |
| | cam19 | SCAAtssfyw | ycttapy...nvWG | QGTQVTVSS |
| | cam20 | YCAIteiewy | gcnlrtrtf..trWG | QGTQVTVSS |
| | cam21 | YCAAnqlagg | wyldpnywls vgay..aiWG | QGTHVTVSS |
| 25 | cam24 | YCAArItemg | acdarwatla trtfaynyWG | RGTQVTVSS |
| | cam25 | YCAVdgwtrk | eggiglpwsv qcedgynyWG | QGTQVTVSS |
| | cam27 | YCKIdsyph | ll.....dvWG | QGTQVTVSS |
| | cam29 | YCAPvey pia | dmcs.....ryGD | PGTQVTVSS |

30

For example, according to Pessi *et al.* (1993) a subdomain portion of a V_H region of
common antibodies (containing both heavy chains and light chains) is sufficient to
direct its folding, provided that a cognate V_L moiety is present. Thus it might be
35 expected from literature on the common antibodies that without V_L chains proper
folding of heavy chains cannot be achieved. A striking difference between the
common antibodies and the *Camelidae*-derived heavy chain antibodies is, that the
highly conserved apolar amino acid leucine (L) at place 45 present in common
antibodies is replaced in most of the *Camelidae*-derived heavy chain antibodies by
40 the charged amino acid arginine (R), thereby preventing binding of the variable
region of the heavy chain to that of the light chains.

Another remarkable feature is that one of the CDRs of the heavy chains of this type
of immunoglobulins from *Camelidae*, CDR3, is often much longer than the

corresponding CDR3 of common heavy chains. Besides the two conserved cysteines forming a disulphide bridge in common V_H fragments, the *Camelidae* V_H fragments often contain two additional cysteine residues, one of which often is present in CDR3.

- 5 According to the present inventors these features indicate that CDR3 may play an important role in the binding of antigens by these heavy chain antibodies and can compensate for the absence of light chains (also containing CDRs) in binding of antigens by immunoglobulins in *Camelidae*.

Thus, as the heavy chains of *Camelidae* do not have special features for interacting
10 with corresponding light chains (which are absent), these heavy chains are very different from common heavy chains of immunoglobulins and seem intrinsically more suitable for secretion by prokaryotic and lower eukaryotic cells.

The present inventors realized that these features make both intact heavy chain
15 immunoglobulins of *Camelidae* and fragments thereof very attractive for their production by microorganisms. The same holds for derivatives thereof including functionalized fragments. In this specification the term "functionalized fragment" is used for indicating an antibody or fragment thereof to which one or more functional groups, including enzymes and other binding polypeptides, are attached resulting in
20 fusion products of such antibody fragment with another biofunctional molecule.

Summary of the invention

In a broad sense the invention provides a process for the production of an antibody or a fragment or functionalized fragment thereof using a transformed lower
25 eukaryotic host containing an expressible DNA sequence encoding the antibody or (functionalized) fragment thereof, wherein the antibody or (functionalized) fragment thereof is derived from a heavy chain immunoglobulin of *Camelidae* and is devoid of light chains, and wherein the lower eukaryotic host is a mould or a yeast. Thus the lower eukaryotic host can be a mould, e.g. belonging to the genera *Aspergillus* or
30 *Trichoderma*, or a yeast, preferably belonging to the yeast genera *Saccharomyces*, *Kluyveromyces*, *Hansenula*, or *Pichia*. Preferably the fragments still contain the whole variable domain of these heavy chains.

The invention also provides methods to produce such heavy chain immunoglobulins or (functionalized) fragments thereof in which methods the framework or the CDRs of these heavy chains are modified by random or directed mutagenesis in such a way that the mutated heavy chain is optimized for secretion by the host microorganism
5 into the fermentation medium.

Another embodiment of the invention is that CDRs can be grafted on these optimized frameworks (compare grafting of CDRs on human immunoglobulins as described by e.g. Jones *et al.*, Nature 321 (1986) 522). These CDRs can be obtained from common antibodies or they may originate from heavy chain immunoglobulins
10 of *Camelidae*. The binding properties may be optimized by random or directed mutagenesis. Thus in a process according to the invention an antibody or (functionalized) fragment thereof derived from a heavy chain immunoglobulin of *Camelidae* can be produced which comprises a CDR different from the CDR belonging to the natural antibody ex *Camelidae* which is grafted on the framework
15 of the variable domain of the heavy chain immunoglobulin ex *Camelidae*.

The invention also provides a method for the microbiological production of catalytic antibodies. These antibodies are preferably raised in *Camelidae* against transition state molecules following procedures similar to the one described by Lerner *et al.*, Science 252 (1991) 659-667. Using random or site-directed mutagenesis such
20 catalytic antibodies or fragments thereof can be modified in such a way that the catalytic activity of these (functionalized) antibodies or fragments can be further improved.

For preparing modified heavy chain antibodies a process according to the invention is provided, in which the DNA sequence encodes a modified heavy chain immunoglobulin or a (functionalized) fragment thereof derived from *Camelidae* and being
25 devoid of light chains, and is made by random or directed mutagenesis or both. Thus the resulting immunoglobulin or (functionalized) fragment thereof is modified such that

- it is better adapted for production by the host cell, or
- 30 - it is optimized for secretion by the lower eukaryotic host into the fermentation medium, or
- its binding properties (k_{on} and k_{off}) are optimized, or

- its catalytic activity is improved, or
- it has acquired a metal chelating activity, or
- its physical stability is improved.

5 Another particular embodiment of the present invention relates to genes encoding fusion proteins consisting of both a heavy chain immunoglobulin from *Camelidae* or part thereof and a second protein or another polypeptide, e.g. an enzyme, in particular an oxido-reductase, and to expression products of such genes. By means of the heavy chain immunoglobulin (fragment) the protein or enzyme can be guided to a
10 target thereby increasing the local efficiency of the protein or enzyme significantly. Thus according to this embodiment of the invention a process is provided, in which the functionalized antibody or fragment thereof comprises a fusion protein of both a heavy chain immunoglobulin from *Camelidae* or a fragment thereof and another polypeptide, e.g. an enzyme, preferably an oxido-reductase.

15

As a result of a process according to the invention known products may be produced, e.g. antibodies also produced by *Camelidae*, but many of the possible products will be new products, thus the invention also provides new products obtainable by a process according to the invention.

20 The products so produced can be used in compositions for various applications. Therefore, the invention also relates to compositions containing a product produced by a process according to the invention. This holds for both old products and new products.

25 Brief Description of the Figures

Figures 1-4 were already described above in the draft publication.

Figure 1 Characterisation and purification of camel IgG classes on Protein A, Protein G and gel filtration.

Figure 2 Repertoire complexity and antigen binding capacity of camel IgG₁,
30 IgG₂ and IgG₃ analysed by radioimmunoprecipitation (A) or Western blotting (B & C).

- Figure 3 Amino acid sequences of the V_H framework, and hinge/ C_H2 of *Camelus dromedarius* heavy chain immunoglobulins, compared to human (italic) V_H framework (subgroup III) and hinges of human IgG (14); see SEQ. ID. NO: 4-12.
- 5 Figure 4 Schematic representation of the structural organisation of the camel immunoglobulins (adapted from 26).
- Figure 5 DNA and amino acid sequences of the Camel V_H fragments followed by the Flag sequence as present in pB03 (Figure 5A), pB09 (Figure 5B) and pB24 (Figure 5C); see SEQ. ID. NO: 32-37.
- 10 Figure 6 Nucleotide sequence of synthetic DNA fragment cloned into pEMBL9 (Example 1); see SEQ. ID. NO: 38-41.
- Figure 7 Schematic drawing of plasmid pUR4423
- Figure 8 Schematic drawing of plasmid pUR4426
- Figure 9 Schematic drawing of plasmid pUR2778
- 15 Figure 10 Schematic drawing of plasmid pUR4429
- Figure 11 Schematic drawing of plasmid pUR4430
- Figure 12 Schematic drawing of plasmid pUR4445
- Figure 13 Schematic drawing of plasmid pUR4446
- Figure 14 Schematic drawing of plasmid pUR4447
- 20 Figure 15 Schematic drawing of plasmid pUR4451
- Figure 16 Schematic drawing of plasmid pUR4453
- Figure 17 Schematic drawings of plasmids pUR4437 and pUR4438
- Figure 18 Schematic drawings of plasmids pUR4439 and pUR4440
- Figure 19 Nucleotide sequence of synthetic DNA fragment cloned into
- 25 pEMBL9 (Example 6); see SEQ. ID. NO: 42-45.
- Figure 20 Schematic drawing of plasmid pAW14B.
- Figure 21 Western blot analysis of culture medium of *S. cerevisiae* transformants containing pUR4423M (see A) or pUR4425M (see B). Samples were taken after 24 (see 1) or 48 hours (see 2). For pUR4425M two bands were found due to glycosylation of the
- 30 antibody fragment.

Detailed description of the invention

The present invention relates to the production of antibodies or (functionalized) fragments thereof derived from heavy chain immunoglobulins of *Camelidae* by eukaryotes, more in particular by lower eukaryotes such as yeasts and fungi.

- 5 Therefore, mRNA encoding immunoglobulins of *Camelidae* was isolated and transcribed into cDNA according to the procedures described in the above given draft publication and not prior-published European patent application 92402326.0. In each case primers for the PCR reaction directed to the N-terminus of the V_H domain and PCR primers that either hybridize with the C-terminal regions of the
- 10 V_H domain or with the short or large hinge regions as described in the above given draft publication, or with the C-terminal region of the C_H2 or C_H3 domains can be used. In this way structural genes can be obtained encoding the following fragments of heavy chain immunoglobulins of *Camelidae* (Table 2).

15

Table 2. The various forms of immunoglobulins of *Camelidae* that can be expressed in microorganisms.

- a. the variable domain of a heavy chain;
- 20 b. the variable domain and the short hinge of a heavy chain;
- c. the variable domain and the long hinge of a heavy chain;
- d. the variable domain, the C_H2 domain, and either the short or long hinge of a heavy chain;
- e. a complete heavy chain, including either the short or long hinge.

25

According to procedures described in detail in the Examples these cDNAs can be integrated into expression vectors.

- Known expression vectors for *Saccharomyces*, *Kluyveromyces*, *Hansenula*, *Pichia* and *Aspergillus* can be used for incorporating a cDNA or a recombinant DNA according
- 30 to the invention. The resulting vectors contain the following sequences that are required for expression: (a) a constitutive, or preferably an inducible, promoter; (b) a leader or signal sequence; (c) one of the structural genes as described in Table 2

- and (d) a terminator. If the vector is an episomal vector, it preferably comprises an origin of replication as well as a selection marker, preferably a food grade selection marker, (EP-A-487159, UNILEVER / Leenhouts *et al.*). If the vector is an integration vector, then it preferably comprises sequences that ensure integration
- 5 and a selection marker in addition to the sequences required for expression of the structural gene encoding a form of the heavy chain immunoglobulin of *Camelidae* or derivatives thereof. The preferred sequences for integration are sequences encoding ribosomal DNA (WO 91/00920, 1991, UNILEVER / Giuseppin *et al.*) whereas the selection marker will be preferably a food grade marker. —
- 10 For *Saccharomyces* the preferred inducible promoter is the GAL7 promoter (EP-A-0255153, UNILEVER / Fellingner *et al.*); for *Kluyveromyces* the preferred inducible promoter is the inulinase promoter (not yet published EP application 92203932.6, UNILEVER / Toschka & Verbakel, which is incorporated herein by reference); for *Hansenula* or *Pichia* the preferred inducible promoter is the methanol-oxidase
- 15 promoter (Sierkstra *et al.*, Current Genetics 19 (1991) 81-87) and for *Aspergillus* the preferred inducible promoter is the endo-xylanase promoter (not prior-published PCT application PCT/EP 92/02896, UNILEVER / Gouka *et al.*, now publicly available as WO-A-93/12237, which is incorporated herein by reference).
- To achieve efficient secretion of the heavy chain immunoglobulin or parts thereof
- 20 the leader (secretion) sequences of the following proteins are preferred: invertase and α -factor for *Saccharomyces*, inulinase for *Kluyveromyces*, invertase for *Hansenula* or *Pichia* (Sierkstra *et al.*, 1991 *supra*) and either glucoamylase or xylanase for *Aspergillus* (not prior-published PCT application WO-A-93/12237, *supra*). As food-grade selection markers, genes encoding anabolic functions like the leucine2 and
- 25 tryptophan3 are preferred (Giuseppin *et al.* 1991, *supra*). The present invention describes the heterologous production of (functionalized) derivatives or fragments of immunoglobulins in a microorganism, which immunoglobulins in nature occur not as a composite of heavy chains and light chains, but only as a composite of heavy chains. Although the secretion mechanism of mammals and microorganisms is quite
- 30 similar, in details there are differences that are important for developing industrial processes.

To obtain frameworks of the heavy chain immunoglobulins, that are optimally secreted by lower eukaryotes, genes encoding several different heavy chains can be cloned into the coat protein of bacteriophages and subsequently the frameworks of these heavy chain immunoglobulins can be mutated using known PCR technology, e.g. Zhou *et al.*, (1991). Subsequently the mutated genes can be cloned in *Saccharomyces* and *Aspergillus* and the secretion of the mutated genes can be compared with the wild type genes. In this way frameworks optimized for secretion may be selected.

Alternatively these structural genes can be linked to the cell wall anchoring part of cell wall proteins, preferably GPI-linked cell wall proteins of lower eukaryotes, which result in the expression of a chimeric protein on the cell wall of these lower eukaryotes (not prior-published EP application 92202080.5, UNILEVER / Klis *et al.*, now publicly available as International (PCT) patent application WO-A-94/01567, which is incorporated herein by reference).

Both methods have the advantage that the binding parts of the immunoglobulins are well exposed to the surrounding of the cell, microorganism, or phage and therefore can bind antigens optimally. By changing the external conditions the binding rates and dissociation rates of this binding reaction can be influenced. Therefore, these systems are very suitable to select for mutated immunoglobulins that have different binding properties. The mutation of the immunoglobulins can either be obtained by random mutagenesis, or directed mutagenesis based on extensive molecular modelling and molecular dynamical studies.

mRNAs encoding heavy chains of immunoglobulins raised in *Camelidae* against transition state molecules (Lerner *et al.*, 1991 *supra*) can be obtained using standard techniques. The structural genes encoding various forms of immunoglobulins according to the invention as summarized in Table 2 can be cloned into the coat protein of bacteriophages or as fusion with the anchoring part of cell wall proteins and can be tested on the catalytic property. In this way immunoglobulins or parts thereof having catalytic properties can be determined and selected. Genes encoding these selected immunoglobulins or parts thereof can be mutated as described before and recloned in bacteriophages, but preferably cloned as chimeric cell wall bound catalysts in lower eukaryotes. By performing appropriate catalytic assays, catalytic

immunoglobulins or parts thereof with improved catalytic properties can be determined and selected using standard techniques.

An important application of antibodies, especially outside the pharmaceutical industry, will be chimeric proteins consisting of the binding part of antibodies and enzymes. In this way catalytic biomolecules can be designed that have two binding properties, one of the enzyme and the other of the antibody. This can result in enzymes that have superior activity. This can be illustrated with the following examples:

- 10 a. If the substrate of the enzymic reaction is produced by an organism or an enzyme is recognized by the binding domain of the antibody, the local concentration of the substrate will be much higher than for enzymes lacking this binding domain and consequently the enzymic reaction will be improved. In fact this is a mimic of vectorial metabolism in cells (compare e.g. Mitchell, (1979) *Science* 206 1148-1159);
- 15 b. If the substrate of the enzymic reaction is converted into a molecule that kills organisms, then the efficiency and specificity of killing can be increased significantly if the enzyme is equipped with an antibody binding domain that recognizes the target organism (e.g. compare Takahashi *et al.*, (1993) *Science* 259 1460-1463);

20

The invention will be illustrated by the following Examples without being limited thereto. In previously filed Unilever patent specifications several expression vectors were described, e.g. for the yeasts *S. cerevisiae*, *Kluyveromyces*, and *Hansenula*, and the mould *Aspergillus*. Examples of these publications are EP-A-0173378

- 25 (UNILEVER / Ledebner *et al.*), EP-A-0255153, *supra*, and PCT applications WO-A-91/19782 (UNILEVER / van Gorcom *et al.*) and (not prior-published) WO-A-93/12237, *supra*. The genes encoding antibodies or (functionalized) fragments thereof according to the invention can be incorporated into the earlier described expression vectors or derivatives thereof using procedures well known to a skilled person in the art. All techniques used for the manipulation and analysis of nucleic acid materials were performed essentially as described in Sambrook *et al.* (1989)
- 30

(see also ref. 23 of the above given draft publication), except where indicated otherwise.

In the description of the Examples the following endonuclease restriction sites are used:

| | | | | |
|----|-----------------|----------|--------------|---|
| 5 | <i>Afl</i> II | C↓TTAAG | <i>Mlu</i> I | A↓CGCGT |
| | <i>Bsp</i> HI | T↓CATGA | <i>Nco</i> I | C↓CATGG |
| | <i>Bsp</i> HI | T↓CATGA | <i>Not</i> I | GC↓GGCCGC |
| | <i>Bst</i> EII | G↓GTNACC | <i>Nru</i> I | TCG↓CGA |
| | <i>Eag</i> I | C↓GGCCG | <i>Sal</i> I | G↓TCGAC |
| 10 | <i>Eco</i> RI | G↓AATTC | <i>Xho</i> I | C↓TCGAG |
| | <i>Hind</i> III | A↓AGCTT | <i>Bbs</i> I | GAAGAC(N) ₂ ↓ CTTCTG(N') ₆ ↓ |

Example 1 Construction of cassettes encoding V_H fragments originating from *Camelidae*.

For the production of V_H fragments originating from *Camelidae*, the antibody gene fragments were isolated and cloned as described above in the draft publication. The thus obtained gene fragments encode the V_H region, a short or a long hinge region and about 14 amino acids of the C_{H2} region. By using standard molecular biological techniques (e.g. PCR technology), the V_H gene fragments could be subcloned and equipped at their 5'-ends with a gene fragment encoding the *pelB* signal sequence and at their 3'-ends with a gene fragment encoding the Flag tail (13 amino acids). Three of these clones were named pB3, pB9 and pB24 and were deposited at the Centraal Bureau voor Schimmelcultures, Baarn on 20 April 1993 with deposition numbers: CBS 270.93, CBS 271.93 and CBS 272.93, respectively. The DNA and amino acid sequences of the *Camelidae*-V_H fragments followed by the Flag sequence are presented in Figure 5(A-C); see SEQ. ID. NO: 32-37.

1.1 Construction of pUR4421

For the construction of yeast expression plasmids encoding the V_H fragments preceded by the invertase (=SUC2) signal sequence, the α-mating factor prepro-

sequence, or the inulinase signal sequence and followed by either nothing, or a Myc tail or Flag tail, the constructs described below can be prepared.

The multiple cloning site of plasmid pEMBL9 (Denthe *et al.*, 1983) (ranging from the *EcoRI* to the *HindIII* site) was replaced by a synthetic DNA fragment having the nucleotide sequence as indicated in Figure 6; see SEQ. ID. NO: 38-41. The 5'-part of this nucleotide sequence comprises an *EagI* site, the first 4 codons of the *Camelidae* V_H gene fragment and a *XhoI* site coinciding with codons 5 and 6. The 3'-part comprises the last 5 codons of the *Camelidae* V_H gene (encoding VTVSS; see SEQ. ID. NO: 47) part of which coincides partially with a *BstEII* site), eleven codons of the Myc tail, and an *EcoRI* site. The *EcoRI* site, originally present in pEMBL9, is not functional any more, because the 5'- end of the nucleotide sequence contains AATTT instead of AATTC, indicated in Figure 6 as "(*EcoRI*)". The resulting plasmid is called pUR4421.

1.2 Constructs with Flag tail.

After digesting the plasmid pB3 with *XhoI* and *EcoRI*, a DNA fragment of approximately 425 bp was isolated from agarose gel. This fragment codes for a truncated V_H-Flag fragment, missing the first 5 amino acids of the *Camelidae* V_H. The obtained fragment can be cloned into pUR4421. To this end plasmid pUR4421 can be digested with *XhoI* and *EcoRI*, after which the about 4 kb vector fragment can be isolated from an agarose gel. Ligation with the about 425 bp fragment will result in plasmid pUR4421-03F.

1.3 Constructs with Myc tail.

After digesting the plasmid pB3 with *XhoI* and *BstEII*, a DNA fragment of approximately 365 bp was isolated from agarose gel. This fragment codes for a truncated V_H fragment, missing both the first 4 (QVKL; see SEQ. ID. NO: 46) and the last 5 (VTVSS; see SEQ. ID. NO: 47) amino acids of the *Camelidae* V_H fragment.

The obtained fragment was cloned into pUR4421. To this end plasmid pUR4421 was digested with *XhoI* and *BstEII*, after which the about 4 kb vector fragment was isolated from an agarose gel. Ligation with the about 365 bp fragment resulted in

plasmid pUR4421-03M, in which the gene encoding the V_H fragment is reconstituted.

1.4 Constructs encoding V_H only.

- 5 Upon digesting pUR4421-03M or pUR4421-03F with *Bst*EII and *Hind*III, the vector fragments of about 4.4 kb can be isolated from agarose gel and religated in the presence of a synthetic linker peptide having the following sequence:

10
$$\begin{array}{ccc} \text{BstEII} & & \text{HindIII} \\ \text{GTCACCGTCTCCTCATAATGA} & & \\ \text{GCAGAGGAGTATTACTTCGA} & & \end{array}$$
 (see SEQ. ID. NO: 48-49).

In the thus obtained plasmid, pUR4421-03, the Myc tail or Flag tail sequences are removed and the V_H gene fragment is directly followed by a stop codon.

1.5 Other constructs.

- 15 After isolating the gene fragments encoding V_H-hinge-C_H2 fragments as described above in the draft publication, or encoding the intact heavy chain immunoglobulin, it is possible, e.g. by using PCR technology, to introduce an appropriate restriction enzyme recognition site (e.g. *Eco*RI or *Hind*III) downstream of the hinge region, downstream of the C_H2 region, or downstream of the total gene. Upon isolating a
20 *Xho*I-*Eco*RI or *Xho*I-*Hind*III fragment encoding the V_H fragment with a C-terminal extension, the fragment can be cloned into pUR4421 digested with the same restriction enzymes.

In analogy with the construction of pUR4421-03, a number of other constructs can be produced encoding functionalized heavy chain fragments in which a second
25 polypeptide is fused to the C-terminal part of the V_H fragment. Optionally, the V_H fragment and the second polypeptide, e.g. an enzyme, might be connected to each other by a peptide linker.

To this end either the *Bst*EII-*Hind*III fragment or the *Bst*EII-*Eco*RI fragment of either pUR4421-03F or pUR4421-03M has to be replaced by another *Bst*EII-*Hind*III
30 or *Bst*EII-*Eco*RI fragment. The latter new fragment should code for the last amino acids (VTVSS, see SEQ.ID. NO: 47) of the V_H fragment, optionally for a linker peptide, and for the polypeptide of interest e.g. an enzyme. Obviously, the introduction of the DNA fragment should result in an in frame fusion between the

V_H gene fragment and the other DNA sequence encoding the polypeptide of interest.

Alternatively, it is possible to replace the *EagI-XhoI* fragment of pUR4421-03 with
5 another DNA fragment, coding for a polypeptide of interest, optionally for a peptide linker, and for the first 4 (QVKL, see SEQ.ID. NO: 46) amino acids of the V_H fragment, resulting in an in frame fusion with the remaining part of the V_H fragment. In this way, it is possible to construct genes encoding functionalized V_H fragments in which the second polypeptide is fused at the N-terminal part of the V_H
10 fragment, optionally via a peptide linker.

Obviously, it is also possible to construct genes encoding functionalized V_H fragments having a polypeptide fused to the N-terminal as well as fused to the C-terminal end, by combining the above described construction routes.

The polypeptides used to functionalize the V_H fragments might be small, like the
15 Myc and the Flag tails, or intact enzymes, like glucose oxidase, or both.

From all the above described constructs, derived from pUR4421, an appropriate *EagI-HindIII* fragment, encoding the functionalized V_H fragment, can be isolated and cloned into a number of different expression plasmids. Several are exemplified
20 in more detail in the following Examples. Although only the V_H fragments are exemplified, similar constructs can be prepared for the production of larger heavy chain fragments (e.g. V_H-hinge or V_H-hinge-C_H2) or intact heavy chains. The *EagI* site is introduced before the first codon of the V_H fragment, facilitating an in frame fusion with different yeast signal sequences.

25 In particular cases, where additional *EagI* and/or *HindIII* sites are present in the cloned fragments, it is necessary to perform partial digestions with one or both restriction enzymes.

Although the above and following constructions only consider the V_H fragment
30 cloned in pB3, a comparable construction route can be used for the construction of expression plasmids for the production of V_H fragments like V_H-09 and V_H-24, or other V_H fragments.

**Example 2 Construction of *S. cerevisiae* episomal expression plasmids for
 Camelidae V_H.**

For the secretion of recombinant protein from *S. cerevisiae* it is worthwhile to test in parallel the two most frequently applied homologous signal sequences, the SUC2
5 invertase signal sequence and the prepro- α mating factor sequence.

The episomal plasmid pSY1 and pSY16 (Harmsen *et al.*, 1993) contain expression cassettes for the α -galactosidase gene. Both plasmids contain the GAL7 promoter and PGK terminator sequences. pSY1 contains the invertase (SUC2) signal
sequence and pSY16 contains a slightly modified (Harmsen *et al.*, 1993) prepro- α -
10 mating factor signal sequence.

Both plasmids, pSY1 and pSY16 can be digested with *EagI* and *HindIII*, the about 6500 bp long vector backbone of both plasmids can be isolated and subsequently ligated with the *EagI/HindIII* fragments from pUR4421-03F (~465 bp), pUR4421-03M (~455 bp) or pUR4421-03 (~405 bp) (See above).

15 This results in a series of 6 different episomal plasmids for expression in *S. cerevisiae*, containing behind the SUC2- and the α mating factor prepro-sequence the V_H-Flag coding sequence (designated pUR4423F and pUR4426F), the V_H-Myc coding sequence (designated pUR4423M and pUR4426M) or the coding sequence of V_H followed by a stop codon (designated pUR4423, Figure 7 and pUR4426,
20 Figure 8).

Obviously, it is possible to use promoter systems different from the inducible GAL7 promoter, e.g. the constitutive GAPDH promoter.

2.1 Production of V_H-03-myc and V_H-24-myc.

25 After introducing the expression plasmids pUR4423M (coding for V_H-03-myc, preceded by the SUC2-signal sequence) and pUR4425M (coding for V_H-24-myc, preceded by the SUC2-signal sequence) into *S. cerevisiae* via electroporation, transformants were selected from minimal medium agar plates (comprising 0.7 % yeast nitrogen base, 2 % glucose and 2 % agar, supplemented with the essential
30 amino acids and bases).

For the production of antibody fragments the transformants were grown overnight in selective minimal medium (comprising 0.7 % yeast nitrogen base, 2 % glucose,

supplemented with the essential amino acids and bases) and subsequently diluted ten times in YPGal medium (comprising 1 % yeast extract, 2 % bacto pepton and 5 % galactose). After 24 and 48 hours of growth, samples were taken for Western blot analysis (Figure 21). For the immuno detection of the produced V_{II}-myc fragments 5 monoclonal anti-myc antibodies were used.

In essentially the same way comparable results were obtained with a yeast transformed with pUR4424M containing a DNA sequence encoding the V_{II}-09-myc protein.

10 **Example 3 Construction of *S. cerevisiae* multicopy integration vectors for the expression of *Camelidae* V_{II}.**

To combine the benefits of high copy number and mitotically stable expression, the concept of a multicopy integration system into the rDNA locus of lower eukaryotes has already been successfully proven (Giuseppin *et al. supra*).

One of these vectors is pUR2778, a derivative of pUR2774 (Giuseppin *et al. supra*)
15 from which the pol1-S.O. reporter gene sequence was removed (Figure 9).

This integrating plasmid, pUR2778, can be used for integration of *Camelidae* V_H coding sequences, hence the vector can be digested with *Sac*I and *Hind*III after which the 7.3 kb vector fragment can be isolated.

From the in example 2 described pUR4423 or pUR4426 types of plasmids, *SacI*-
20 *HindIII* fragments can be isolated encoding a V_H fragment preceded by a signal
sequence (SUC2 or α mating factor prepro) and followed by nothing or a Myc or
Flag tail.

Ligation of these *SacI-HindIII* fragments with the ~7.3 kb vector fragment will result in integration plasmids, encoding the (functionalized) V_H fragments under the regulation of the strong and inducible GAL7 promoter.

In this way the following expression plasmids were obtained:

| | |
|------------|---|
| pUR4429 | P _{gal7} - SUC2 sig.seq. - V _{II} -03 |
| pUR4429F | P _{gal7} - SUC2 sig.seq. - V _{II} -03 - Flag tail |
| pUR4429M | P _{gal7} - SUC2 sig.seq. - V _{II} -03 - Myc tail |
| pUR4430 | P _{gal7} - α mat.fac. prepro. - V _{II} -03 |
| 5 pUR4430F | P _{gal7} - α mat.fac. prepro. - V _{II} -03 - Flag tail |
| pUR4430M | P _{gal7} - α mat.fac. prepro. - V _{II} -03 - Myc tail |

For schematic drawings see Figure 10 for pUR4429 and Figure 11 for pUR4430.

Obviously, comparable constructs can be prepared for other heavy chain antibodies
 10 or fragments thereof.

As mentioned before, different promoters might be used, for example, the
 constitutive GAPDH promoter.

**Example 4 Construction of expression plasmids for the production of
 15 (functionalized) V_{II} fragments from *Camelidae* by *Kluyveromyces***

**4.1. Construction of *Kluyveromyces lactis* episomal expression plasmids
Camelidae.**

Yeast strains of the genus *Kluyveromyces* have been used for the production of
 enzymes, such as β -galactosidase for many years, and the growth of the strains has
 20 been extensively studied. *Kluyveromyces lactis* is well known for the ability to utilize
 a large variety of compounds as carbon and energy sources for growth. Since these
 strains are able to grow at high temperatures and exhibit high growth rates, they are
 promising hosts for industrial production of heterologous proteins (Hollenberg, C. *et*
al., EP-A-0096430, GIST-BROCADES N.V., 1983).

25 The plasmids pUR2427 and pUR2428 are pTZ19R derivatives with the promoter
 and the DNA sequence encoding either the signal peptide (=pre-sequence) (in
 pUR2428), or the natural prepro-sequence (in pUR2427), of inulinase (inu) from
Kluyveromyces marxianus. Both plasmids contain a unique *Bsp*MI site suitable to
 create a perfect joint with *Eag*I or *Not*I digested DNA-fragments (not yet published
 30 European patent application 92203932.6, *supra*). In both plasmids a unique *Hind*III
 site is located a bit further downstream of the *Bsp*MI-site, so that *Eag*I-*Hind*III cut
 DNA-fragments encoding V_{II} from *Camelidae* either solely or with Myc- or Flag- tail

can be easily ligated into *Bsp*MI-*Hind*III digested pUR2427 or pUR2428. Thereby a set of six plasmids can be created containing the promoter and secretion signals of the *Kluyveromyces marxianus* inulinase gene, joint in frame to *Camelidae* Vh encoding sequences, all on a *Eco*RI-*Hind*III restriction fragment:

- 5 pUR4445 P_{inu} - Inu prepro seq. - V_{II} - 03
- pUR4445M P_{inu} - Inu prepro seq. - V_{II} - 03 - Myc
- pUR4445F P_{inu} - Inu prepro seq. - V_{II} - 03 - Flag
- pUR4446 P_{inu} - Inu pre seq. - V_{II} - 03
- pUR4446M P_{inu} - Inu pre seq. - V_{II} - 03 - Myc
- 10 pUR4446F P_{inu} - Inu pre seq. - V_{II} - 03 - Flag .

Maps of pUR4445 and pUR4446 are shown in Figure 12 and Figure 13.

- The *Eco*RI-*Hind*III fragments of these plasmids can be ligated into the expression vector pSK1 (not yet published European patent application 92203932.6, *supra*),
- 15 from which the α -galactosidase expression cassette including the *GAL7*-promoter is removed with a *Eco*RI(partial) and *Hind*III digestion. The resulting plasmids can then be transformed for example in *K. lactis* strain MSK110 (a, *ura4*, *trp1::URA3*), as they contain the *trp1* marker and the pKD1 episomal plasmid sequences:

- pUR4447 P_{inu} - Inu prepro seq. - V_H - 03
- 20 pUR4447M P_{inu} - Inu prepro seq. - V_H - 03 - Myc
- pUR4447F P_{inu} - Inu prepro seq. - V_H - 03 - Flag
- pUR4448 P_{inu} - Inu pre seq. - V_{II} - 03
- pUR4448M P_{inu} - Inu pre seq. - V_{II} - 03 - Myc
- pUR4448F P_{inu} - Inu pre seq. - V_{II} - 03 - Flag .
- 25 A map of pUR4447 is shown in Figure 14.

Transformation can be performed by standard techniques such as the methods of Beggs (1978) or electroporation, using 0.67% Yeast Nitrogen Base (without amino acids) and 2% glucose as the selection medium for transformants.

4.2. Construction of *Kluyveromyces lactis* multicopy integration vectors.

Alternatively, since all tailed and non-tailed versions of the V_H fragments, joined to the inulinase promoter and secretion signals, are located on *EcoRI-HindIII* fragments, the rDNA multicopy integration plasmid pMIRKGAL-T Δ 1 (Bergkamp *et al.*, 1992) can be used in a similar way as the pSK1 plasmid. In order to replace the α -gal expression cassette present in this plasmid, by a antibody fragment cassette, these plasmids have to be digested with *EcoRI*(partial) and *HindIII*. After isolating the vector fragments, they can be ligated with the about 1.2 kb *EcoRI-HindIII* fragments which can be obtained from the plasmids described in example 4.1. The resulting plasmids can be linearized with *SacII* and transformed to MSK110, resulting in *K. lactis* strains with potentially high and stable expression of single chain V_H fragments.

| | |
|-------------|---|
| pUR4449 | P _{inu} - Inu prepro seq. - V _H - 03 |
| pUR4449M | P _{inu} - Inu prepro seq. - V _H - 03 - Myc |
| 15 pUR4449F | P _{inu} - Inu prepro seq. - V _H - 03 - Flag |
| pUR4450 | P _{inu} - Inu pre seq. - V _H - 03 |
| pUR4450M | P _{inu} - Inu pre seq. - V _H - 03 - Myc |
| pUR4450F | P _{inu} - Inu pre seq. - V _H - 03 - Flag . |

20 4.3. Construction of *Kluyveromyces marxianus* episomal plasmids.

Kluyveromyces marxianus is a yeast which is perhaps even more attractive than *K. lactis* for industrial biotechnology, due to its short generation time on glucose (about 45 minutes) and its ability to grow on a wide range of substrates, and its growth at elevated temperatures (Rouwenhorst *et al.*, 1988).

25 The shuttle vector pUR2434, containing the leu2 marker and the pKD1 plasmid sequences (not yet published European patent application 92203932.6, *supra*), located on a pUC19 based vector, can be cut with *EcoRI*(partial) and *HindIII* to remove the α -galactosidase expression cassette. In this vector the *EcoRI-HindIII* fragments containing the V_H expression cassettes as described in example 4.1, can be
30 ligated. The resulting plasmids can then be transformed into KMS3, the neat leu2-auxotroph CBS6556 *K. marxianus* strain (Bergkamp, 1993) using the method of Meilhoc *et al.* (1990).

- pUR4451 P_{inu} - Inu prepro seq. - V_{II} - 03
 pUR4451M P_{inu} - Inu prepro seq. - V_{II} - 03 - Myc
 pUR4451F P_{inu} - Inu prepro seq. - V_{II} - 03 - Flag
 pUR4452 P_{inu} - Inu pre seq. - V_{II} - 03
 5 pUR4452M P_{inu} - Inu pre seq. - V_{II} - 03 - Myc
 pUR4452F P_{inu} - Inu pre seq. - V_{II} - 03 - Flag .

A map of pUR4451 is shown in Figure 15.

4.4 Construction of *Kluyveromyces marxianus* multicopy integration vectors.

- 10 For high and stable expression in *Kluyveromyces marxianus*, the multicopy integration system as described by Bergkamp (1993), can be used. The following cloning route, based on the route for constructing pMIRKM-GAL5 (Bergkamp, 1993), results in suitable expression vectors for production of Vh fragments from *Camelidae*. The *EcoRI-NheI*(Klenow filled) fragments of pUR4447,-M,-F and pUR4448,-M,-F
 15 containing the Vh fragment expression cassettes as described in example 4.1, can be isolated and ligated in *EcoRI-EcoRV* digested pIC-20H. From the plasmids obtained in this way, and which are equivalents of the pIC- α gal plasmid, the *BamHI-NruI* fragment can be isolated and ligated with *BamHI-SmaI* digested pMIRKM4. The result of this will be expression vectors which are equivalent to pMIRKM-GAL5,
 20 and contain a tailed or non-tailed Vh fragment from camel under control of inulinase promoter and secretion signals, in a vector which also contains the *K. marxianus* *LEU2*-gene with defective promoter, and *K. marxianus* rDNA sequences for targeted integration into the genome. These vectors can be used to transform for example KMS3.
- 25 pUR4453 P_{inu} - Inu prepro seq. - V_{II} - 03
 pUR4453M P_{inu} - Inu prepro seq. - V_{II} - 03 - Myc
 pUR4453M P_{inu} - Inu prepro seq. - V_{II} - 03 - Flag
 pUR4454 P_{inu} - Inu pre seq. - V_{II} - 03
 pUR4454M P_{inu} - Inu pre seq. - V_{II} - 03 - Myc
 30 pUR4454F P_{inu} - Inu pre seq. - V_{II} - 03 - Flag .

A map of pUR4453 is shown in Figure 16.

Example 5. Construction of *Hansenula polymorpha* integrating vectors for the expression of (functionalized) V_H fragments from *Camelidae*.

In search for productive systems able to carry out authentic posttranscriptional processing and overcoming the limitation of higher eukaryotic expression systems, such as high costs, low productivity and the need for stringent control procedures for the detection of contaminating agents could be overcome by the methylotrophic yeast *H. polymorpha*. This strain is able to grow on methanol as its sole carbon and energy source, so the presence of methanol in the growth medium rapidly induces the enzymes of the methanol pathway, such as the key enzymes methanol oxidase (MOX) and dihydroxyacetone synthase (DHAS).

While experiments to express foreign genetic information from an episomal plasmid resulted in a low plasmid stability, chromosomal integration is the method of choice (Sierkstra *et al.*, 1991). By utilizing the DNA of the *mox* gene as integration locus the latter were able to express and secrete α -galactosidase regulated by *mox* promoter and -terminator. Here, the *S. cerevisiae* SUC2 signal sequence was proven to be efficiently functional for secretion.

The same approach can be used for expression and secretion of *Camelidae* V_H antibody fragments. Plasmids analogous to pUR3515 (without an origin of replication functional in yeast) and pUR3517 (containing the HARS2 sequence as origin of replication) can be used as expression vectors (Sierkstra *et al.*, 1991). As a starting vector pUR3501 can be used (Sierkstra *et al.*, 1991) in which by means of site directed mutagenesis (e.g. via PCR technology), an *EagI* restriction site is introduced at the junction between the invertase (=SUC2) signal sequence and the α -galactosidase. From the resulting plasmid, pUR3501*Eag*, it is possible to replace the *EagI*-*HindIII* fragment comprising the α -galactosidase gene by an *EagI*-*HindIII* fragment encoding a (functionalized) antibody fragment, obtained as described in example 1. In case of using the *EagI*-*HindIII* fragments of the pUR4421-03 series (example 1), this would result in plasmids pUR4437 (Figure 17), pUR4437M and pUR4437F. In these plasmids the nucleotide sequence encoding the (functionalized) V_H is preceded by a nucleotide sequence encoding the invertase signal sequence and the *mox* promoter sequence. The obtained plasmids can be digested with *Bam*HI and *Hind*III and after filling in the sticky ends with Klenow polymerase, the about

2.6 kb fragments can be ligated into plasmid pUR3511 which was digested with *Sma*I (Sierkstra *et al.*, 1991). In this way the terminator sequence of the *mox* gene can be fused downstream of the V_{II} encoding sequences. From the thus obtained plasmids, pUR4438 (Figure 17) *Eco*RI-*Hind*III fragments of about 3 kb can be

5 isolated, containing the *mox* promoter, the invertase signal sequence, the (functionalized) V_{II} fragment and the *mox* transcription terminator. Subsequently these fragments can be cloned into plasmid pUR3513 (no yeast origin of replication) or in pUR3514 (HARS origin of replication) as described by Sierkstra *et al.* (1991), resulting in two sets of plasmids:

10

| | |
|-------------|---|
| pUR4439 | P_{mox} - SUC2 sig. seq. - V_{II} - <i>mox</i> term. -- no origin |
| pUR4439M | P_{mox} - SUC2 sig. seq. - V_{II} - <i>mox</i> term. -- no origin |
| pUR4439F | P_{mox} - SUC2 sig. seq. - V_{II} - <i>mox</i> term. -- no origin |
| pUR4440 | P_{mox} - SUC2 sig. seq. - V_{II} - <i>mox</i> term. -- HARS origin |
| 15 pUR4440M | P_{mox} - SUC2 sig. seq. - V_{II} - <i>mox</i> term. -- HARS origin |
| pUR4440F | P_{mox} - SUC2 sig. seq. - V_{II} - <i>mox</i> term. -- HARS origin . |

Maps of pUR4439 and pUR4440 are shown in Figure 18.

Essentially the same can be done with other *Eag*I-*Hind*III fragment, obtained as

20 described in example 1.

The newly obtained plasmids can be transformed by electroporation of *H. polymorpha* A16 (CBS4732, *leu*-) and can be selected by growing on selective medium containing 0.68% YNB and 2% glucose. Induction medium should contain 0.5% methanol instead of the glucose.

25

Example 6 Construction *Aspergillus niger* var. *awamori* integration vectors for the production of V_{II} fragments from *Camelidae*.

The multiple cloning site of plasmid pEMBL9 (ranging from the *Eco*RI to the *Hind*III site) was replaced by a synthetic DNA fragment having the nucleotide

30 sequence as indicated in Figure 19; see SEQ. ID. NO: 42-45. The 5'- part of the nucleotide sequence contains a *Nru*I restriction site followed by the first codons of the *Camelidae* V_{II} gene fragment and a *Xho*I restriction site. The 3'-part encodes for

pUR4433F or pUR4433M with other *BstEII-AflIII* fragments, resulting in frame fusions encoding functionalized V_H fragments, having a C-terminal extension.

Upon replacing the *NruI-XhoI* fragments of pUR4433, pUR4433F or pUR4433M, in frame fusions can be constructed encoding functionalized V_H fragments, having an

5 N-terminal extension.

In the above described constructs an *NruI* site was introduced before the first codon of the (functionalized) V_H fragment, facilitating an in frame fusion with the precursor-sequence of xylanase, see (not prior-published) WO-A-93/12237, *supra*.

For the construction of *Aspergillus* expression plasmids, from the plasmids

10 pUR4433F, pUR4433M and pUR4433, respectively, an about 455, 445 and 405 bp *NruI-AflIII* fragment has to be isolated encoding the V_H fragment with a Flag, a Myc or no tail.

Plasmid pAW14B was the starting vector for construction of a series of expression
15 plasmids containing the *exlA* expression signals and the genes coding for (functionalized) V_H fragments of *Camelidae* heavy chain antibodies. The plasmid comprises an *Aspergillus niger* var. *awamori* chromosomal 5 kb *SalI* fragment on which the 0.7 kb *exlA* gene is located, together with 2.5 kb of 5'-flanking sequences and 2.0 kb of 3'-flanking sequences (see Figure 20 and (not prior-published) WO-A-
20 93/12237, *supra*).

Starting from pAW14B, pAW14B-10 was constructed by removing the *EcoRI* site originating from the pUC19 polylinker, and introducing a *NotI* site. This was achieved by digesting plasmid pAW14B with *EcoRI* and after dephosphorylation the linear 7.9 kb *EcoRI* fragment was isolated. The fragment was religated in the
25 presence of the "*EcoRI*"-*NotI* linker:

5'- AATTGCGGCCGC -3'

(see SEQ. ID. NO: 52).

Subsequently the *AflIII* site, located downstream of the *exlA* terminator was removed by partially cleaving plasmid pAW14B-10 and religating the isolated, linearized plasmid after filling in the sticky ends, resulting in plasmid

30 pAW14B-11.

Finally, pAW14B-12 was constructed using pAW14B-11 as starting material. After digestion of pAW14B-11 with *AflIII* (overlapping with the *exlA* stop codon) and *BglIII*

(located in the *exI* promoter) the ~2.4 kb *Afl*III-*Bgl*III fragment, containing part of the *exI*A promoter and the *exI*A gene was isolated as well as the ~5.5 kb *Afl*III-*Bgl*III vector fragment. After partial digestion of this ~2.4 kb fragment with *Bsp*HI (located in the *exI*A promoter and at the *exI*A start codon) an about 1.8 kb *Bgl*III-*Bsp*HI *exI*A promoter fragment (up to the ATG initiation codon) was isolated and ligated with the about 5.5 kb *Afl*III-*Bgl*III vector fragment of pAW14B-11 in the presence of the following adaptor:

10 (BspHI) BbsI AflII
CATGCAGTCTTCGGGC
GTCAGAAGCCCGAATT (see SEQ. ID. NO: 53-54).

For the construction of the V_H expression plasmids, pAW14B-11 can be partially digested with *Nru*I and digested with *Afl*II, after which the ~ 7 kb vector fragment can be isolated from agarose gel and contains the xylanase promoter, the DNA sequence encoding the xylanase signal sequence and the xylanase terminator. Upon ligation of the *Nru*I-*Afl*II fragments of pUR4433M, pUR4434M and pUR4435M with the pAW14B-11 vector, plasmids pUR4436M, pUR4437M and pUR4438M were obtained, respectively. In these plasmids the *Camelidae* V_H polypeptides are preceded by the 27 amino acid long precursor sequence of xylanase and followed by the myc-tail (of 11 amino acids; see Examples 1.3 en 2, Figures 6 and 19, and SEQ.ID. NO: 41 = 45).

In a similar way plasmids can be constructed encoding the V_H fragments followed by the FLAG-tail or without a tail.

After introducing the *amdS* and *pyrG* selection markers into the unique *NotI* site of pUR4436M, pUR4437M and pUR4438M using conventional techniques, e.g. as
25 described in Examples 2 and 3 of (not prior-published) WO-A-93/12237, *supra*, the plasmids were transferred to *Aspergillus*.

Production of the Camel V_{II} fragments by the selected transformants was achieved by growing the strains in inducing medium essentially as described in example 2.2 of (not prior-published) WO-A-93/12237, *supra*. Western blot analysis of the culture medium was performed as described in Example 2.1 above and revealed the presence of the antibody fragments.

Obviously, expression vectors can be constructed in which different promoter systems, e.g. glucoamylase promoter, and/or different signal sequences, e.g. glucoamylase or glucose oxidase signal sequences, are used.

5 Example 7 Production of glucose oxidase - V_{II} fusion proteins

Glucose oxidase catalyses the oxidation of D-glucose to D-gluconate under the release of hydrogen peroxide. Glucose oxidase genes (*gox*) from *Aspergillus niger* have been cloned (Frederick *et al.* (1990) J. Biol. Chem. 265 3793, Kriechbaum *et al.*, 1989) and the nucleotide sequences are available from the EMBL data bank
10 under accession numbers J05242 and X16061. The nucleotide sequence of the latter is used as a basis for the following construction route.

Upon cloning the *gox* gene from *A. niger* it is possible, by applying PCR technology, to introduce convenient restriction sites.

To introduce a *Bsp*HI restriction site, overlapping with the ATG initiation codon,
15 the sequence ATC ATG CAG can be changed to ATC ATG AGG. In the same experiment an *Eco*RI restriction site can be introduced which is located upstream of the *Bsp*HI site. This can be achieved by using the following PCR primer:

*Eco*RI *Bsp*HI

5'-TCACTGAATTCGGGATC ATG AGG ACT CTC CTT GTG AGC TCG CTT-3'

20 (see SEQ. ID. NO: 55).

A second PCR primer, having the following sequence can be used:

*Afl*III *Bbs*I *Sal*I

5'-ATGTCACAAAGCTTAAGCACGAAGACA GTC GAC CGT GCG GCC GGA GAC-3'

*Hind*III

25 (see SEQ. ID. NO: 56)

in the same PCR experiment, in order to introduce a *Bbs*I site, a *Afl*III site and a *Hind*III site, downstream of the unique *Sal*I site present in the glucose oxidase gene. After digesting the DNA obtained from this PCR experiment with *Eco*RI and *Hind*III, an *Eco*RI - *Hind*III fragment of about 160 bp can be isolated and cloned
30 into pEMBL9, which was digested with the same enzymes, resulting in plasmid pGOX1.

From pGOX1 an about 140 bp *Bsp*HI - *Afl*III fragment can be isolated and introduced into the 7.2 kb *Bbs*I-*Afl*III vector fragment of pAW14B-12, resulting in

pAW14B-GOX. In this plasmid, the 5'- part of the *gox* gene, encoding the first 43 amino acids, is fused in frame with the ATG initiation codon of the *exlA* gene.

In a second PCR experiment, a *MluI* restriction site can be introduced near the 3'-
 5 end of the *gox* by changing the sequence TAT GCT TCC to TAC GCG TCC. In the same experiment a *HindIII* site can be introduced downstream of the *MluI* site. As a second primer an oligo nucleotide should be used hybridizing upstream of the *Sall* site. After digesting the DNA obtained from this PCR experiment with *Sall* and
 — *HindIII*, an *Sall* - *HindIII* fragment of about 1.7 kb can be isolated and cloned into
 10 pEMBL9, which was digested with the same enzymes, resulting in plasmid pGOX2. Upon digesting pGOX2 with *MluI* and *HindIII*, an about 5.7 kb vector fragment can be isolated.

From the plasmids pUR4433, pUR4433F, pUR4433M and the like, *XhoI*-*HindIII* fragments can be isolated, encoding the truncated *Camelidae* V_H fragment with or
 15 without a tail sequence, and missing the first 4-6 N-terminal amino acids (see Example 1). These fragments can be ligated into the 5.7 kb pGOX2 vector fragment by using *MluI*-*XhoI* adaptors. These adaptors are designed in such a way that they result in an in frame fusion between the 3'-end of the *gox* gene and the restored V_H gene fragment, optionally intersected with a DNA sequence encoding a peptide
 20 linker sequence.

An example of these designed adaptors is:

| | |
|---|-----------------------------------|
| <i>MluI</i> | <i>XhoI</i> |
| <u>CGCGTCCATGCAGTCCTCAGGTGGATCATCCCAGGTGAAACTGC</u> | |
| AGGTACGTCAGGAGTCCACCTAGTAGGGTCCACTTTGACGAGCT | |
| 25 | S M Q S S G G S S Q V K L L E |

(see SEQ. ID. NO: 57-59)

which encodes for the last amino acids of GOX, an SSGGSS linker sequence (see
 SEQ. ID. NO: 62) and the N-terminal amino acids of the Camel V_H fragment of
 pB3. Instead of the SSGGSS linker (see SEQ. ID. NO: 62) it is possible to use other
 30 linkers such as the repeated sequence linkers described in the above indicated European patent application 92402326.0, e.g. a repeated sequence Pro-X, with X being any amino acid, but preferably Gln, Lys or Glu, the sequence containing

advantageously at least 3 repeats of Pro-X and especially a fragment composed of a 12-fold repeat of the sequence Pro-X.

- In case the about 435 bp *XhoI-HindIII* fragment of pUR4433M is used in combination with the above described adaptor, this would result in pGOX2-03M. From this plasmid a *Sall-AflII* fragment of about 2.1 kb encoding the C-terminal part of glucose oxidase followed by the linker peptide, the Camel V_H fragment of pB3 and finally the Myc tail.
- Upon digesting pAW14B-GOX partially with *BbsI*, and with *AflII*, the about 7.4 kb vector fragment can be isolated. This fragment contains the xylanase promoter, the DNA sequence encoding the N-terminal part of glucose oxidase and the xylanase promoter. Due to the digestion with *BbsI*, a *Sall* sticky end is created, corresponding with the *Sall* restriction site originally present in the *gox* gene. Ligation of the *Sall-AflII* vector fragment with the about 2.1 kb *Sall-AflII* fragment of pGOX2-03M, resulting in pUR4441M. This expression plasmid encodes for a single chain polypeptide comprising the glucose oxidase enzyme, the (functionalized) Camel V_H fragment and the Myc tail.
- Introduction of this type of expression plasmids in *Aspergillus* can be achieved essentially as described in example 6.
- As the naturally occurring glucose oxidase is a homodimeric enzyme, it might be expected that a fusion protein, comprising glucose oxidase and an antibody fragment as a C-terminal extension, has an increased avidity for the antigen/antibody binding, if this fusion protein is produced as a homodimer. Alternatively, it is possible to produce heterodimers, consisting of one glucose oxidase molecule connected to a V_H fragment and one wild type glucose oxidase molecule. This can be achieved by producing with the same strain both wild type glucose oxidase and the fused glucose oxidase-V_H fragment, or by mixing the two different homodimers produced by different strains under conditions whereby the mixture of dimers are dissociated and subsequently associated.

Example 8 Engineering of *Camelidae* V_H fragments

8.1 Random and targeted random mutagenesis.

After expressing a number of different *Camelidae* V_H fragments in lower eukaryotic host organisms as described above, or in prokaryotes, fragments produced in relative
 5 higher amounts can be selected. Upon subjecting the *Xho*I-*Bst*EII gene fragments to a (targeted) random mutagenesis procedure, it might be possible to further improve special characteristics of the V_H fragment, e.g. further improvement of the production level, increased stability or increased affinity.

To this end the following procedure might be followed.

- 10 Upon replacing the polylinker of the phagemid vector pHEN1 (Hoogenboom *et al.*, 1991) located on a *Nco*I-*Not*I fragment by a new polylinker having the following sequence:

| | | | | | | | |
|----|---|--|--------------|--|----------------|--|--------------|
| | <i>Nco</i> I | | <i>Xho</i> I | | <i>Bst</i> EII | | <i>Not</i> I |
| 15 | <u>CATGGCCAGGTGAAACTGCTCGAGTAAGTGACTAAGGTCACCGTCTCCTCAGC</u> CGGTCCACTTTGACGAGCTCATTCACTGATTCCAGTGGCAGAGGAGTCGCCGG | | | | | | |

(see SEQ. ID. NO: 60-61) it becomes possible to introduce *Xho*I-*Bst*EII fragments encoding truncated *Camelidae* V_H fragments in the phagemid.

- Following mutagenesis of the V_H encoding sequence (random mutagenesis) or a
 20 specific part thereof (targeted random mutagenesis), the mutated V_H fragments can be expressed and displayed on the phage surface in essentially the same way as described by Hoogenboom *et al.* (1991). Selecting phages displaying (mutant) V_H fragments, can be done in different ways, a number of which are described by Marks *et al.* (1992). Subsequently, the mutated *Xho*I-*Bst*EII fragments can be isolated from
 25 the phagemid and introduced into expression plasmids for yeast or fungi as described in previous examples.

Upon producing the mutant V_H fragments by these organisms, the effects of the mutations on production levels, V_H fragment stability or binding affinity can be evaluated easily and improved V_H fragments can be selected.

- 30 Obviously, a similar route can be followed for larger antibody fragments. With similar procedures the activity of catalytic antibodies can be improved.

8.2 Site-directed or designed mutagenesis

As an alternative to the methods described above in Example 8.1 it is possible to use the well-known technique of site-directed mutagenesis. Thus, designed mutations, preferably based on molecular modelling and molecular dynamics, can be introduced in the V_H fragments, e.g. in the framework or in the CDRs.

8.3 Construction V_H fragments with regulatable binding efficiencies.

For particular applications, the possibility to regulate the binding capacity of antibody fragments might be necessary. The introduction of metal ion binding sites in proteins is known from the literature e.g. Pessi *et al.* (1993). The present inventors envisage that the introduction of a metal binding site in an antibody fragment by rational design can result in a regulatable antibody fragment, when the metal binding site is introduced at a position such that the actual binding of the metal ion results in a conformational change in the antibody fragments due to which the binding of the antigen to the antibody fragment is influenced. Another possibility is that the presence of the metal prevents antigen binding due to steric hindrance.

8.4 Grafting of CDR regions on the framework fragments of a Camelidae V_H fragment.

Grafting of CDR fragments onto framework fragments of different antibodies or fragments thereof is known from the literature (see Jones *et al.* (1986), WO-A-92/15683, and WO-A-92/01059). In these cases the CDR fragments of murine antibody fragments were grafted onto framework fragments of human antibodies. The sole rationale behind the "humanization" was to increase the acceptability for therapeutic and/or diagnostic applications in human.

Essentially the same approach can however also be used for a totally different purpose. Although antibody fragments share some homology in the framework areas, the production levels vary considerably.

Once an antibody or an antibody fragment, e.g. a Camelidae V_H fragment, has been identified, which can be produced to high levels by a production organism of interest, this antibody (fragment) can be used as a starting point to construct "grafted" antibody (fragments), which can be produced in high levels and have an

other specificity as compared to the original antibody (fragment). In particular cases it might be necessary to introduce some modifications in the framework fragments as well in order to obtain optimal transitions between the framework fragments and the CDR fragments. For the determination of the optimal transitions molecular dynamics and molecular modelling can be used.

To this end a synthetic gene, encoding the "grafted V_H" fragment, can be constructed and introduced into an expression plasmid. Obviously it is possible to adapt the codon usage to the codons preferred by the host organism.

For optimization of the "grafted V_H" fragment, the procedure as described in example 8.1 can be followed.

Literature mentioned in the specification additional to that mentioned in the above given draft publication

- Adair, J.R. *et al.*, WO-A-92/01059 (CELLTECH Ltd, 1992)
- 15 - Beggs (1978) *Nature* 275 104
- Bendig, M.M. *et al.* WO-A-92/15683 (MERCK PATENT GmbH, 1992)
- Bergkamp, R.J.M., Kool, I.M., Geerse, R.H., Planta, R.J. (1992) Multiple copy integration of the α -galactosidase gene from *Cyamopsis tetragonoloba* into the ribosomal DNA of *Kluyveromyces lactis*. *Current Genetics* 21 365-370
- 20 - Bergkamp, R.J.M., PhD Thesis Free University of Amsterdam (1993), Heterologous gene expression in *Kluyveromyces* yeasts
- Better *et al.* (1988) *Science* 240 1041-1043
- Bird *et al.*, (1988) *Science* 242 423-426
- Cabilly, S. *et al.*, EP-A-0125023 (GENENTECH, 1984)
- 25 - Denthe, *et al.* (1983) *Nucl. Acids Res.* 11 1645
- Fellingner, A.J. *et al.*, EP-A-0255153 (UNILEVER, 1988)
- Frederick *et al.* (1990) *J. Biol. Chem.* 265 3793
- Giuseppin, M.L.F., Lopes, M.T.S., Planta, R.J., Verbakel, J.M.A., Verrips, C.T. (1991) Process for preparing a protein by a yeast transformed by multicopy integration of an expression vector. PCT application WO 91/00920 (UNILEVER)
- 30 - Harmsen, M.M., Langedijk, A.C., van Tuinen, E., Geerse, R.H., Rauè, H.A., Maat, J., (1993) Effect of *pmr1* disruption and different signal sequences on the

- intracellular processing and secretion of *Cyamopsis tetragonoloba* α -galactosidase by *S. cerevisiae*. *Gene* **125** 115-123
- Hollenberg, C. *et al.*, EP-A-0096430 (GIST-BROCADES N.V., 1983))
 - Hoogenboom H.R., Griffiths, A.D., Johnson, K.S., Chiswell, D.J., Hudson, P., and
 - 5 Winter, G. (1991) Multi-subunit proteins on the surface of filamentous phage: methodologies for displaying antibody (Fab) heavy and light chains. *Nucleic Acids Research* **15** 4133-4137
 - Jones *et al.* (1986) *Nature* **321** 522
 - Kriechbaum *et al.* (1989) *FEBS Lett.* **255** 63
 - 10 - Ledebøer, A.M. *et al.*, EP-A-0173378 (UNILEVER, 1986)
 - Leenhouts, C.J. *et al.*, EP-A-0487159 (UNILEVER, 1992)
 - Lerner, Benkovic and Schultz, (1991) *Science* **252** 659-667
 - Marks, J.D., Hoogenboom, H.R., Griffiths, A.D., and Winter, G. (1992) Molecular evolution of proteins on filamentous phage. *J. Biol. Chem.* **267** 16007-16010
 - 15 - Meilhoc, E., Masson, J., Teissié, J. (1990) High efficiency transformation of intact yeast cells by electric pulses. *Bio/Technology* **8** 223-227
 - Mitchell, P., (1979) *Science* **206** 1148-1159)
 - Pessi *et al.* (1993) *Nature* **362** 367.
 - Rouwenhorst, R.J., Visser, L.E., van der Baan, Scheffers, W.A., van Dijken, J.P.
 - 20 (1988) Production, distribution and kinetic properties of inulinase in continuous culture of *Kluyveromyces marxianus* CBS 6556. *Appl. Environm. Microbiol.* **54** 1131-1137.
 - Sierkstra, L.N., Verbakel, J.M.A. and Verrips, C.T. (1991) Optimisation of a host/vector system for heterologous gene expression by *Hansenula polymorpha*.
 - 25 *Current Genetics* **19** 81-87.
 - Skerra *et al.* (1988) *Science* **240** 1938
 - Takahashi *et al.* (1993) *Science* **259** 1460-1463);
 - Teeri *et al.*, WO-A-93/02198 (TECH. RES. CENT. FINLAND, publ. 04.02.1993)
 - Van Gorcom, R.F.M. *et al.*, WO-A-91/19782 (UNILEVER, 1991)
 - 30 - Wu *et al.* (1993) *Bio/Technology* **11** 71
 - Zhou *et al.* (1991) *Nucleic Acids Research* **19** 6052

Additional references to prior-filed but not prior-published patent applications, which are incorporated herein by reference:

- not prior-published PCT application EP 92/02896, filed 09.12.92 with priority date of 09.12.91 (UNILEVER / R.J. Gouka *et al.*), now publicly available as
5 WO-A-93/12237
- not prior-published EP application 92202080.5, filed 08.07.92 (UNILEVER / F.M. Klis *et al.*), now publicly available as International (PCT) patent application WO-A-94/01567)
- not prior-published EP application 92402326.0, filed 21.08.92 (C. Casterman & R.
10 Hamers), now publicly available as EP-A1-0 584 421
- not yet published EP application 92203932.6, filed 11.12.92 (UNILEVER / H.Y. Toschka & J.M.A. Verbakel).

15

Information on deposits of micro-organisms under the Budapest Treaty is given in Example 1 on page 23, lines 23-25 above. In agreement with Rule 28 (4) EPC, or a
20 similar arrangement for a State not being a Contracting State of the EPC, it is hereby requested that a sample of such deposit, when requested, will be submitted to an expert only.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- 5 (i) APPLICANT:
- (A) NAME: Unilever N.V.
(B) STREET: Weena 455
(C) CITY: Rotterdam
(E) COUNTRY: The Netherlands
10 (F) POSTAL CODE (ZIP): NL-3013 AL
- (A) NAME: Unilever PLC
(B) STREET: Unilever House Blackfriars
(C) CITY: London
15 (E) COUNTRY: United Kingdom
(F) POSTAL CODE (ZIP): EC4P 4BQ
- (A) NAME: Leon Gerardus Joseph FRENKEN
(B) STREET: Geldersestraat 90
(C) CITY: Rotterdam
20 (E) COUNTRY: The Netherlands
(F) POSTAL CODE (ZIP): NL-3011 MP
- (A) NAME: Cornelis Theodorus VERRIPS
(B) STREET: Hagedoorn 18
(C) CITY: Maassluis
25 (E) COUNTRY: The Netherlands
(F) POSTAL CODE (ZIP): NL-3142 KB
- (A) NAME: Raymond HAMERS
(B) STREET: Vijversweg 15
(C) CITY: Sint-Genesius-Rode
30 (E) COUNTRY: Belgium
(F) POSTAL CODE (ZIP): B-1640
- (A) NAME: Cécle HAMERS-CASTERMAN
(B) STREET: Vijversweg 15
(C) CITY: Sint-Genesius-Rode
35 (E) COUNTRY: Belgium
(F) POSTAL CODE (ZIP): B-1640
- (A) NAME: Serge Victor Marie MUYLDERMANS
(B) STREET: Brusselse Steenweg 55
(C) CITY: Hoeilaart
40 (E) COUNTRY: Belgium
(F) POSTAL CODE (ZIP): B-1560
- 45 (ii) TITLE OF INVENTION: Production of antibodies or (functionalized)
50 fragments thereof derived from heavy chain immunoglobulins
of Camelidae.
- (iii) NUMBER OF SEQUENCES: 62
- (iv) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Floppy disk
55 (B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)
- 60 (2) INFORMATION FOR SEQ ID NO: 1:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 5 amino acids
65 (B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

5 Ala Pro Glu Leu Leu
 1 5

(2) INFORMATION FOR SEQ ID NO: 2:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

 Ala Pro Glu Leu Pro
 1 5

25 (2) INFORMATION FOR SEQ ID NO: 3:

 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: DNA (genomic)

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

 CGCCATCAAG GTACCA GTTG A

40 (2) INFORMATION FOR SEQ ID NO: 4:

 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 89 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: protein

50 (vii) IMMEDIATE SOURCE:
 (B) CLONE: human heavy chain framework (subgroup III)
 (Xaa = CDR1, Xaa Xaa = CDR2 and Xaa Xaa Xaa = CDR3)

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

60 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Xaa Trp Val Arg Gln Ala
 20 25 30

 Pro Gly Lys Gly Leu Glu Trp Val Ser Xaa Xaa Arg Phe Thr Ile Ser
 35 40 45

65 Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg
 50 55 60

Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Xaa Xaa Xaa Trp Gly
 65 70 75 80

5 Gln Gly Thr Leu Val Thr Val Ser Ser
 85

(2) INFORMATION FOR SEQ ID NO: 5:

- 10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 81 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 15 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

20 (B) CLONE: camel "heavy chain immunoglobulin" framework A
 (Xaa = CDR1, Xaa Xaa = CDR2 and Xaa Xaa Xaa = CDR3)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

25 Gly Gly Ser Val Gln Gly Gly Gly Ser Leu Arg Leu Ser Cys Ala Ile
 1 5 10 15
 Ser Gly Xaa Trp Phe Arg Glu Gly Pro Gly Lys Glu Arg Glu Gly Ile
 20 25 30
 30 Ala Xaa Xaa Arg Phe Thr Ile Ser Gln Asp Ser Thr Leu Lys Thr Met
 35 40 45
 Tyr Leu Leu Met Asn Asn Leu Lys Pro Glu Asp Thr Gly Thr Tyr Tyr
 50 55 60
 35 Cys Ala Ala Xaa Xaa Xaa Trp Gly Gln Gly Thr Gln Val Thr Val Ser
 65 70 75 80
 40 Ser

(2) INFORMATION FOR SEQ ID NO: 6:

- 45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 81 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 50 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

55 (B) CLONE: camel "heavy chain immunoglobulin" framework B
 (Xaa = CDR1, Xaa Xaa = CDR2 and Xaa Xaa Xaa = CDR3)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

60 Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Ala Ser
 1 5 10 15
 Ser Ser Xaa Trp Tyr Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
 20 25 30
 65 Ser Xaa Xaa Arg Phe Thr Ile Ser Gln Asp Ser Ala Lys Asn Thr Val
 35 40 45

Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr
 50 55 60
 5 Cys Lys Ile Xaa Xaa Xaa Trp Gly Gln Gly Thr Gln Val Thr Val Ser
 65 70 75 80
 Ser

10 (2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 37 amino acids
 (B) TYPE: amino acid
 15 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20 (vii) IMMEDIATE SOURCE:

(B) CLONE: camel "heavy chain immunoglobulin"
framework - short hinge - CH2 fragment

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Thr Asn Glu Val
 1 5 10 15
 30 Cys Lys Cys Pro Lys Cys Pro Ala Pro Glu Leu Pro Gly Gly Pro Ser
 20 25 30
 Val Phe Val Phe Pro
 35

35

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 60 amino acids
 40 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: camel "heavy chain immunoglobulin"
framework - long hinge - CH2 fragment

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Glu Pro Lys Ile Pro
 1 5 10 15
 55 Gln Pro Gln Pro Lys Pro Gln Pro Gln Pro Gln Pro Gln Pro Lys Pro
 20 25 30
 Gln Pro Lys Pro Glu Pro Glu Cys Thr Cys Pro Lys Cys Pro Ala Pro
 35 40 45
 60 Glu Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro
 50 55 60

65

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 67 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: human gamma-3 CH1 - hinge - CH2 fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

15 Lys Val Asp Lys Arg Val Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr
 1 5 10 15
 20 His Thr Cys Pro Arg Cys Pro Glu Pro Lys Cys Ser Asp Thr Pro Pro
 20 25 30
 Pro Cys Pro Arg Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro
 35 40 45
 25 Cys Pro Arg Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
 50 55 60
 Leu Phe Pro
 65

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 35 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: human gamma-1 CH1 - hinge - CH2 fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

45 Lys Val Asp Lys Lys Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr
 1 5 10 15
 50 Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
 20 25 30
 Leu Phe Pro
 35

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- 60 (A) LENGTH: 31 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: human gamma-2 CH1 - hinge - CH2 fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

5

Lys Val Lys Val Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro
 1 5 10 15

10

Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro
 20 25 30

(2) INFORMATION FOR SEQ ID NO: 12:

15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: human gamma-4 CH1 - hinge - CH2 fragment

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser
 1 5 10 15

30

Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
 20 25 30

35

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 121 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: protein

45

(vii) IMMEDIATE SOURCE:

(B) CLONE: mouse heavy chain V-region

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

50

Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Thr Phe Ser Asp Phe
 20 25 30

55

Tyr Met Glu Trp Val Arg Gln Pro Pro Gly Lys Arg Leu Glu Trp Ile
 35 40 45

60

Ala Ala Ser Arg Asn Lys Ala Asn Asp Tyr Thr Thr Glu Tyr Ser Ala
 50 55 60

Ser Val Lys Gly Arg Phe Ile Val Ser Arg Asp Thr Ser Gln Ser Ile
 65 70 75 80

65

Leu Tyr Leu Gln Met Asn Ala Leu Arg Ala Glu Asp Thr Ala Ile Tyr
 85 90 95

Tyr Cys Ala Arg Asp Tyr Tyr Gly Ser Ser Tyr Phe Asp Val Trp Gly
 100 105 110

5 Ala Gly Thr Thr Val Thr Val Ser Ser
 115 120

(2) INFORMATION FOR SEQ ID NO: 14:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 131 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 15 (ii) MOLECULE TYPE: protein
 (vii) IMMEDIATE SOURCE:
 (B) CLONE: human heavy chain V-region
 20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:
 25 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 30 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Xaa Ile Ser Xaa Lys Thr Asp Gly Gly Xaa Thr Tyr Tyr Ala Asp
 50 55 60
 35 Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
 65 70 75 80
 Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
 40 85 90 95
 Tyr Cys Ala Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Tyr
 100 105 110
 45 Tyr Tyr Tyr His Xaa Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
 115 120 125
 Val Ser Ser
 130

50

(2) INFORMATION FOR SEQ ID NO: 15:

55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 114 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 60 (ii) MOLECULE TYPE: protein
 (vii) IMMEDIATE SOURCE:
 (B) CLONE: camel "heavy chain immunoglobulin" V-region (1)
 65 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:
 Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala
 1 5 10 15

Ser Gly Tyr Ser Asn Cys Pro Leu Thr Trp Ser Trp Tyr Arg Gln Phe
 20 25 30
 5 Pro Gly Thr Glu Arg Glu Phe Val Ser Ser Met Asp Pro Asp Gly Asn
 35 40 45
 Thr Lys Tyr Thr Tyr Ser Val Lys Gly Arg Phe Thr Met Ser Arg Gly
 50 55 60
 10 Ser Thr Glu Tyr Thr Val Phe Leu Gln Met Asp Asn Leu Lys Pro Glu
 65 70 75 80
 Asp Thr Ala Met Tyr Tyr Cys Lys Thr Ala Leu Gln Pro Gly Gly Tyr
 85 90 95
 15 Cys Gly Tyr Gly Xaa Cys Leu Trp Gly Gln Gly Thr Gln Val Thr Val
 100 105 110
 20 Ser Ser

(2) INFORMATION FOR SEQ ID NO: 16:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 120 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 30 (ii) MOLECULE TYPE: protein
 (vii) IMMEDIATE SOURCE:
 (B) CLONE: camel "heavy chain immunoglobulin" V-region (2)
 35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:
 Asp Val Gln Leu Val Ala Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
 1 5 10 15
 40 Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Asp Ser Phe Ser Arg Phe
 20 25 30
 Ala Met Ser Trp Phe Arg Gln Ala Pro Gly Lys Glu Cys Glu Leu Val
 35 40 45
 45 Ser Ser Ile Gln Ser Asn Gly Arg Thr Thr Glu Ala Asp Ser Val Gln
 50 55 60
 50 Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Arg Asn Thr Val Tyr Leu
 65 70 75 80
 Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Gly
 85 90 95
 55 Ala Val Ser Leu Met Asp Arg Ile Ser Gln His Gly Cys Arg Gly Gln
 100 105 110
 Gly Thr Gln Val Thr Val Ser Leu
 115 120

(2) INFORMATION FOR SEQ ID NO: 17:

65 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 123 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

5 (vii) IMMEDIATE SOURCE:

(B) CLONE: camel "heavy chain immunoglobulin" V-region (3)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

10 Gly Gly Ser Val Gln Thr Gly Gly Ser Leu Arg Leu Ser Cys Ala Val
1 5 10 15
Ser Gly Phe Ser Phe Ser Thr Ser Cys Met Ala Trp Phe Arg Gln Ala
20 25 30
15 Ser Gly Lys Gln Arg Glu Gly Val Ala Ala Ile Asn Ser Gly Gly Gly
35 40 45
Arg Thr Tyr Tyr Asn Thr Tyr Val Ala Glu Ser Val Lys Gly Arg Phe
50 55 60
20 Ala Ile Ser Gln Asp Asn Ala Lys Thr Thr Val Tyr Leu Asp Met Asn
65 70 75 80
25 Asn Leu Thr Pro Glu Asp Thr Ala Thr Tyr Tyr Cys Ala Ala Val Pro
85 90 95
Ala His Leu Gly Pro Gly Ala Ile Leu Asp Leu Lys Lys Tyr Lys Tyr
100 105 110
30 Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

35 (2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 116 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

45 (vii) IMMEDIATE SOURCE:

(B) CLONE: camel "heavy chain immunoglobulin" V-region (7)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

50 Gly Gly Ser Val Gln Gly Gly Gly Ser Leu Arg Leu Ser Cys Ala Ile
1 5 10 15
Ser Gly Tyr Thr Tyr Gly Ser Phe Cys Met Gly Trp Phe Arg Glu Gly
20 25 30
55 Pro Gly Lys Glu Arg Glu Gly Ile Ala Thr Ile Leu Asn Gly Gly Thr
35 40 45
60 Asn Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Gln
50 55 60
Asp Ser Thr Leu Lys Thr Met Tyr Leu Leu Met Asn Asn Leu Lys Pro
65 70 75 80
65 Glu Asp Thr Gly Thr Tyr Tyr Cys Ala Ala Glu Leu Ser Gly Gly Ser
85 90 95

Cys Glu Leu Pro Leu Leu Phe Asp Tyr Trp Gly Gln Gly Thr Gln Val
 100 105 110

Thr Val Ser Ser
 115

5

(2) INFORMATION FOR SEQ ID NO: 19:

10

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 114 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

20

(B) CLONE: camel "heavy chain immunoglobulin" V-region (9)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

25

Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Thr Leu Ser Cys Val Tyr
 1 5 10 15

Thr Asn Asp Thr Gly Thr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys
 20 25 30

30

Glu Cys Glu Arg Val Ala His Ile Thr Pro Asp Gly Met Thr Phe Ile
 35 40 45

Asp Glu Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Gln
 50 55 60

35

Lys Thr Leu Ser Leu Arg Met Asn Ser Leu Arg Pro Glu Asp Thr Ala
 65 70 75 80

Val Tyr Tyr Cys Ala Ala Asp Trp Lys Tyr Trp Thr Cys Gly Ala Gln
 85 90 95

40

Thr Gly Gly Tyr Phe Gly Gln Trp Gly Gln Gly Ala Gln Val Thr Val
 100 105 110

Ser Ser

45

(2) INFORMATION FOR SEQ ID NO: 20:

50

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 125 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: camel "heavy chain immunoglobulin" V-region (11)

60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Asn Val
 1 5 10 15

65

Ser Gly Ser Pro Ser Ser Thr Tyr Cys Leu Gly Trp Phe Arg Gln Ala
 20 25 30

56

Pro Gly Arg Glu Arg Glu Gly Val Thr Ala Ile Asn Thr Asp Gly Ser
 35 40 45
 5 Ile Ile Tyr Ala Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Gln
 50 55 60
 Asp Thr Ala Lys Glu Thr Val His Leu Gln Met Asn Asn Leu Gln Pro
 65 70 75 80
 10 Glu Asp Thr Ala Thr Tyr Tyr Cys Ala Ala Arg Leu Thr Glu Met Gly
 85 90 95
 Ala Cys Asp Ala Arg Trp Ala Thr Leu Ala Thr Arg Thr Phe Ala Tyr
 100 105 110
 15 Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
 115 120 125

20 (2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 114 amino acids
 25 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: protein
 30 (vii) IMMEDIATE SOURCE:
 (B) CLONE: camel "heavy chain immunoglobulin" V-region (13)
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:
 35 Gly Gly Ser Val Glu Ala Gly Gly Ser Leu Arg Leu Ser Cys Thr Ala
 1 5 10 15
 Ser Gly Tyr Val Ser Ser Met Ala Trp Phe Arg Gln Val Pro Gly Gln
 20 25 30
 40 Glu Arg Glu Gly Val Ala Phe Val Gln Thr Ala Asp Asn Ser Ala Leu
 35 40 45
 45 Tyr Gly Asp Ser Val Lys Gly Arg Phe Thr Ile Ser His Asp Asn Ala
 50 55 60
 Lys Asn Thr Leu Tyr Leu Gln Met Arg Asn Leu Gln Pro Asp Asp Thr
 65 70 75 80
 50 Gly Val Tyr Tyr Cys Ala Ala Gln Lys Lys Asp Arg Thr Arg Trp Ala
 85 90 95
 Glu Pro Arg Glu Trp Asn Asn Trp Gly Gln Gly Thr Gln Val Thr Ala
 100 105 110
 55 Ser Ser

60 (2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 122 amino acids
 65 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: camel "heavy chain immunoglobulin" V-region (16)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

5 Gly Gly Ser Ala Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala
1 5 10 15

10 His Gly Ile Pro Leu Asn Gly Tyr Tyr Ile Ala Trp Phe Arg Gln Ala
20 25 30

Pro Gly Lys Gly Arg Glu Gly Val Ala Thr Ile Asn Gly Gly Arg Asp
35 40 45

15 Val Thr Tyr Tyr Ala Asp Ser Val Thr Gly Arg Phe Thr Ile Ser Arg
50 55 60

Asp Ser Pro Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro
65 70 75 80

20 Glu Asp Thr Ala Ile Tyr Phe Cys Ala Ala Gly Ser Arg Phe Ser Ser
85 90 95

25 Pro Val Gly Ser Thr Ser Arg Leu Glu Ser Ser Asp Tyr Asn Tyr Trp
100 105 110

Gly Gln Gly Ile Gln Val Thr Ala Ser Ser
115 120

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: camel "heavy chain immunoglobulin" V-region (17)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

45 Gly Gly Ser Val Gln Pro Gly Gly Ser Leu Thr Leu Ser Cys Thr Val
1 5 10 15

50 Ser Gly Ala Thr Tyr Ser Asp Tyr Ser Ile Gly Trp Ile Arg Gln Ala
20 25 30

Pro Gly Lys Asp Arg Glu Val Val Ala Ala Ala Asn Thr Gly Ala Thr
35 40 45

55 Ser Lys Phe Tyr Val Asp Phe Val Lys Gly Arg Phe Thr Ile Ser Gln
50 55 60

60 Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln Met Ser Phe Leu Lys Pro
65 70 75 80

Glu Asp Thr Ala Ile Tyr Tyr Cys Ala Ala Ala Asp Pro Ser Ile Tyr
85 90 95

65 Tyr Ser Ile Leu Xaa Ile Glu Tyr Lys Tyr Trp Gly Gln Gly Thr Gln
100 105 110

Val Thr Val Ser Ser
115

5 (2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 123 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

15 (vii) IMMEDIATE SOURCE:

(B) CLONE: camel "heavy chain immunoglobulin" V-region (18)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

20 Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Thr Gly
1 5 10 15
Ser Gly Phe Pro Tyr Ser Thr Phe Cys Leu Gly Trp Phe Arg Gln Ala
25 20 25 30
Pro Gly Lys Glu Arg Glu Gly Val Ala Gly Ile Asn Ser Ala Gly Gly
35 35 40 45
Asn Thr Tyr Tyr Ala Asp Ala Val Lys Gly Arg Phe Thr Ile Ser Gln
30 50 55 60
Gly Asn Ala Lys Asn Thr Val Phe Leu Gln Met Asp Asn Leu Lys Pro
65 70 75 80
35 Glu Asp Thr Ala Ile Tyr Tyr Cys Ala Ala Asp Ser Pro Cys Tyr Met
85 90 95
Pro Thr Met Pro Ala Pro Pro Ile Arg Asp Ser Phe Gly Trp Asp Asp
40 100 105 110
Phe Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

45 (2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

- 50 (A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

55 (vii) IMMEDIATE SOURCE:

(B) CLONE: camel "heavy chain immunoglobulin" V-region (19)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

60 Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala
1 5 10 15
Ser Asp Tyr Thr Ile Thr Asp Tyr Cys Met Ala Trp Phe Arg Gln Ala
65 20 25 30
Pro Gly Lys Glu Arg Glu Leu Val Ala Ala Ile Gln Val Val Arg Ser
35 40 45

Asp Thr Arg Leu Thr Asp Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr
 50 55 60
 5 Ile Ser Gln Gly Asn Thr Lys Asn Thr Val Asn Leu Gln Met Asn Ser
 65 70 75 80
 Leu Thr Pro Glu Asp Thr Ala Ile Tyr Ser Cys Ala Ala Thr Ser Ser
 85 90 95
 10 Phe Tyr Trp Tyr Cys Thr Thr Ala Pro Tyr Asn Val Trp Gly Gln Gly
 100 105 110
 Thr Gln Val Thr Val Ser Ser
 115
 15

(2) INFORMATION FOR SEQ ID NO: 26:

20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 117 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 25 (ii) MOLECULE TYPE: protein
 (vii) IMMEDIATE SOURCE:
 (B) CLONE: camel "heavy chain immunoglobulin" V-region (20)
 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:
 Gly Gly Ser Val Gln Val Gly Gly Ser Leu Arg Leu Ser Cys Val Ala
 1 5 10 15
 35 Ser Thr His Thr Asp Ser Ser Thr Cys Ile Gly Trp Phe Arg Gln Ala
 20 25 30
 Pro Gly Lys Glu Arg Glu Gly Val Ala Ser Ile Tyr Phe Gly Asp Gly
 35 40 45
 40 Gly Thr Asn Tyr Arg Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Gln
 50 55 60
 45 Leu Asn Ala Gln Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro
 65 70 75 80
 Glu Asp Ser Ala Met Tyr Tyr Cys Ala Ile Thr Glu Ile Glu Trp Tyr
 85 90 95
 50 Gly Cys Asn Leu Arg Thr Thr Phe Thr Arg Trp Gly Gln Gly Thr Gln
 100 105 110
 Val Thr Val Ser Ser
 115
 55

(2) INFORMATION FOR SEQ ID NO: 27:

60 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 125 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 65 (ii) MOLECULE TYPE: protein

60

(vii) IMMEDIATE SOURCE:

(B) CLONE: camel "heavy chain immunoglobulin" V-region (21)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

5 Gly Gly Ser Val Gln Val Gly Gly Ser Leu Lys Leu Ser Cys Lys Ile
1 5 10 15

10 Ser Gly Gly Thr Pro Asp Arg Val Pro Lys Ser Leu Ala Trp Phe Arg
20 25 30

Gln Ala Pro Glu Lys Glu Arg Glu Gly Ile Ala Val Leu Ser Thr Lys
35 40 45

15 Asp Gly Lys Thr Phe Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile
50 55 60

Phe Leu Asp Asn Asp Lys Thr Thr Phe Ser Leu Gln Leu Asp Arg Leu
65 70 75 80

20 Asn Pro Glu Asp Thr Ala Asp Tyr Tyr Cys Ala Ala Asn Gln Leu Ala
85 90 95

25 Gly Gly Trp Tyr Leu Asp Pro Asn Tyr Trp Leu Ser Val Gly Ala Tyr
100 105 110

Ala Ile Trp Gly Gln Gly Thr His Val Thr Val Ser Ser
115 120 125

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 125 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: camel "heavy chain immunoglobulin" V-region (24)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

45 Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Asn Val
1 5 10 15

50 Ser Gly Ser Pro Ser Ser Thr Tyr Cys Leu Gly Trp Phe Arg Gln Ala
20 25 30

Pro Gly Lys Glu Arg Glu Gly Val Thr Ala Ile Asn Thr Asp Gly Ser
35 40 45

55 Val Ile Tyr Ala Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Gln
50 55 60

60 Asp Thr Ala Lys Lys Thr Val Tyr Leu Gln Met Asn Asn Leu Gln Pro
65 70 75 80

Glu Asp Thr Ala Thr Tyr Tyr Cys Ala Ala Arg Leu Thr Glu Met Gly
85 90 95

65 Ala Cys Asp Ala Arg Trp Ala Thr Leu Ala Thr Arg Thr Phe Ala Tyr
100 105 110

Asn Tyr Trp Gly Arg Gly Thr Gln Val Thr Val Ser Ser
 115 120 125

5 (2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 129 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

15 (vii) IMMEDIATE SOURCE:

(B) CLONE: camel "heavy chain immunoglobulin" V-region (25)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

20 Gly Gly Ser Val Gln Thr Gly Gly Ser Leu Arg Leu Ser Cys Glu Ile
 1 5 10 15
 Ser Gly Leu Thr Phe Asp Asp Ser Asp Val Gly Trp Tyr Arg Gln Ala
 20 25 30
 25 Pro Gly Asp Glu Cys Lys Leu Val Ser Gly Ile Leu Ser Asp Gly Thr
 35 40 45
 30 Pro Tyr Thr Lys Ser Gly Asp Tyr Ala Glu Ser Val Arg Gly Arg Val
 50 55 60
 Thr Ile Ser Arg Asp Asn Ala Lys Asn Met Ile Tyr Leu Gln Met Asn
 65 70 75 80
 35 Asp Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Ala Val Asp Gly
 85 90 95
 Trp Thr Arg Lys Glu Gly Gly Ile Gly Leu Pro Trp Ser Val Gln Cys
 100 105 110
 40 Glu Asp Gly Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
 115 120 125
 45 Ser

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

- 50 (A) LENGTH: 111 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: camel "heavy chain immunoglobulin" V-region (27)

60 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Ala Ser
 1 5 10 15
 65 Ser Ser Lys Tyr Met Pro Cys Thr Tyr Asp Met Thr Trp Tyr Arg Gln
 20 25 30

62

Ala Pro Gly Lys Glu Arg Glu Phe Val Ser Ser Ile Asn Ile Asp Gly
 35 40 45
5 Lys Thr Thr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Gln
 50 55 60
Asp Ser Ala Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro
65 70 75 80
10 Glu Asp Thr Ala Met Tyr Tyr Cys Lys Ile Asp Ser Tyr Pro Cys His
 85 90 95
Leu Leu Asp Val Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
15 100 105 110

(2) INFORMATION FOR SEQ ID NO: 31:

- 20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 112 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- 25 (ii) MOLECULE TYPE: protein
- (vii) IMMEDIATE SOURCE:
 (B) CLONE: camel "heavy chain immunoglobulin" V-region (29)

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Val Ala
 1 5 10 15
35 Ser Gly Phe Asn Phe Glu Thr Ser Arg Met Ala Trp Tyr Arg Gln Thr
 20 25 30
Pro Gly Asn Val Cys Glu Leu Val Ser Ser Ile Tyr Ser Asp Gly Lys
40 35 40 45
Thr Tyr Tyr Val Asp Arg Met Lys Gly Arg Phe Thr Ile Ser Arg Glu
50 55 60
45 Asn Ala Lys Asn Thr Leu Tyr Leu Gln Leu Ser Gly Leu Lys Pro Glu
65 70 75 80
Asp Thr Ala Met Tyr Tyr Cys Ala Pro Val Glu Tyr Pro Ile Ala Asp
 85 90 95
50 Met Cys Ser Arg Tyr Gly Asp Pro Gly Thr Gln Val Thr Val Ser Ser
 100 105 110

55 (2) INFORMATION FOR SEQ ID NO: 32:

- 55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 416 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 60 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vii) IMMEDIATE SOURCE:
 65 (B) CLONE: camel "heavy chain immunoglobulin" V-region followed
 by the FLAG sequence (pB03)

63

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..408

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

| | | |
|----|---|-----|
| | CAG GTG AAA CTG CTC GAG TCT GGG GGA GGC TCG GTG CAG GCT GGG GGG | 48 |
| | Gln Val Lys Leu Leu Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly | |
| 1 | 1 5 10 15 | |
| 10 | TCT CTG ACA CTC TCT TGT GTA TAC ACC AAC GAT ACT GGG ACC ATG GGA | 96 |
| | Ser Leu Thr Leu Ser Cys Val Tyr Thr Asn Asp Thr Gly Thr Met Gly | |
| | 20 25 30 | |
| 15 | TGG TTT CGC CAG GCT CCA GGG AAA GAG TGC GAA AGG GTC GCG CAT ATT | 144 |
| | Trp Phe Arg Gln Ala Pro Gly Lys Glu Cys Glu Arg Val Ala His Ile | |
| | 35 40 45 | |
| 20 | ACG CCT GAT GGT ATG ACC TTC ATT GAT GAA CCC GTG AAG GGG CGA TTC | 192 |
| | Thr Pro Asp Gly Met Thr Phe Ile Asp Glu Pro Val Lys Gly Arg Phe | |
| | 50 55 60 | |
| 25 | ACG ATC TCC CGA GAC AAC GCC CAG AAA ACG TTG TCT TTG CGA ATG AAT | 240 |
| | Thr Ile Ser Arg Asp Asn Ala Gln Lys Thr Leu Ser Leu Arg Met Asn | |
| | 65 70 75 80 | |
| 30 | AGT CTG AGG CCT GAG GAC ACG GCC GTG TAT TAC TGT GCG GCA GAT TGG | 288 |
| | Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala Asp Trp | |
| | 85 90 95 | |
| 35 | AAA TAC TGG ACT TGT GGT GCC CAG ACT GGA GGA TAC TTC GGA CAG TGG | 336 |
| | Lys Tyr Trp Thr Cys Gly Ala Gln Thr Gly Gly Tyr Phe Gly Gln Trp | |
| | 100 105 110 | |
| 40 | GGT CAG GGG GCC CAG GTC ACC GTC TCC TCA CTA GCT AGT TAC CCG TAC | 384 |
| | Gly Gln Gly Ala Gln Val Thr Val Ser Ser Leu Ala Ser Tyr Pro Tyr | |
| | 115 120 125 | |
| 45 | GAC GTT CCG GAC TAC GGT TCT TAATAGAATT C | 416 |
| | Asp Val Pro Asp Tyr Gly Ser | |
| | 130 135 | |

45 (2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 135 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

| | |
|----|---|
| 55 | Gln Val Lys Leu Leu Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly |
| | 1 5 10 15 |
| | Ser Leu Thr Leu Ser Cys Val Tyr Thr Asn Asp Thr Gly Thr Met Gly |
| | 20 25 30 |
| 60 | Trp Phe Arg Gln Ala Pro Gly Lys Glu Cys Glu Arg Val Ala His Ile |
| | 35 40 45 |
| 65 | Thr Pro Asp Gly Met Thr Phe Ile Asp Glu Pro Val Lys Gly Arg Phe |
| | 50 55 60 |

Thr Ile Ser Arg Asp Asn Ala Gln Lys Thr Leu Ser Leu Arg Met Asn
 65 70 75 80
 5 Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala Asp Trp
 85 90 95
 Lys Tyr Trp Thr Cys Gly Ala Gln Thr Gly Gly Tyr Phe Gly Gln Trp
 100 105 110
 10 Gly Gln Gly Ala Gln Val Thr Val Ser Ser Leu Ala Ser Tyr Pro Tyr
 115 120 125
 Asp Val Pro Asp Tyr Gly Ser
 130 135
 15

(2) INFORMATION FOR SEQ ID NO: 34:

- 20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 443 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 25 (ii) MOLECULE TYPE: DNA (genomic)
 (vii) IMMEDIATE SOURCE:
 (B) CLONE: camel "heavy chain immunoglobulin" V-region followed
 30 by the FLAG sequence (pB09)
 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..435
 35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

CAG GTG AAA CTG CTC GAG TCT GGA GGA GGC TCG GTG CAG ACT GGA GGA 48
 Gln Val Lys Leu Leu Glu Ser Gly Gly Gly Ser Val Gln Thr Gly Gly
 1 5 10 15
 40 TCT CTG AGA CTC TCC TGT GCA GTC TCT GGA TTC TCC TTT AGT ACC AGT 96
 Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Phe Ser Phe Ser Thr Ser
 20 25 30
 45 TGT ATG GCC TGG TTC CGC CAG GCT TCA GGA AAG CAG CGT GAG GGG GTC 144
 Cys Met Ala Trp Phe Arg Gln Ala Ser Gly Lys Gln Arg Glu Gly Val
 35 40 45
 50 GCA GCC ATT AAT AGT GGC GGT GGT AGG ACA TAC TAC AAC ACA TAT GTC 192
 Ala Ala Ile Asn Ser Gly Gly Gly Arg Thr Tyr Tyr Asn Thr Tyr Val
 50 55 60
 55 GCC GAG TCC GTG AAG GGC CGA TTC GCC ATC TCC CAA GAC AAC GCC AAG 240
 Ala Glu Ser Val Lys Gly Arg Phe Ala Ile Ser Gln Asp Asn Ala Lys
 65 70 75 80
 ACC ACG GTA TAT CTT GAT ATG AAC AAC CTA ACC CCT GAA GAC ACG GCT 288
 Thr Thr Val Tyr Leu Asp Met Asn Asn Leu Thr Pro Glu Asp Thr Ala
 85 90 95
 60 ACG TAT TAC TGT GCG GCG GTC CCA GCC CAC TTG GGA CCT GGC GCC ATT 336
 Thr Tyr Tyr Cys Ala Ala Val Pro Ala His Leu Gly Pro Gly Ala Ile
 100 105 110
 65 CTT GAT TTG AAA AAG TAT AAG TAC TGG GGC CAG GGG ACC CAG GTC ACC 384
 Leu Asp Leu Lys Lys Tyr Lys Tyr Trp Gly Gln Gly Thr Gln Val Thr
 115 120 125

GTC TCC TCA CTA GCT AGT TAC CCG TAC GAC GTT CCG GAC TAC GGT TCT 432
 Val Ser Ser Leu Ala Ser Tyr Pro Tyr Asp Val Pro Asp Tyr Gly Ser
 130 135 140

5 TAATAGAATT C

145

443

10 (2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 144 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

1 Gln Val Lys Leu Leu Glu Ser Gly Gly Gly Ser Val Gln Thr Gly Gly
 5 10 15
 25 Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Phe Ser Phe Ser Thr Ser
 20 25 30
 Cys Met Ala Trp Phe Arg Gln Ala Ser Gly Lys Gln Arg Glu Gly Val
 35 40 45
 30 Ala Ala Ile Asn Ser Gly Gly Gly Arg Thr Tyr Tyr Asn Thr Tyr Val
 50 55 60
 35 Ala Glu Ser Val-Lys Gly Arg Phe Ala Ile Ser Gln Asp Asn Ala Lys
 65 70 75 80
 Thr Thr Val Tyr Leu Asp Met Asn Asn Leu Thr Pro Glu Asp Thr Ala
 85 90 95
 40 Thr Tyr Tyr Cys Ala Ala Val Pro Ala His Leu Gly Pro Gly Ala Ile
 100 105 110
 Leu Asp Leu Lys Lys Tyr Lys Tyr Trp Gly Gln Gly Thr Gln Val Thr
 115 120 125
 45 Val Ser Ser Leu Ala Ser Tyr Pro Tyr Asp Val Pro Asp Tyr Gly Ser
 130 135 140

50 (2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 449 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

(B) CLONE: camel heavy chain immunoglobulin" V-region followed
 by the FLAG sequence (pB24)

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..441

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

66

| | | |
|----|--|-----|
| | CAG GTG AAA CTG CTC GAG TCT GGG GGA GGG TCG GTG CAG GCT GGA GGG Gln Val Lys Leu Leu Glu Ser Gly Gly Ser Val Gln Ala Gly Gly | 48 |
| | 1 5 10 15 | |
| 5 | TCT CTG AGA CTC TCC TGT AAT GTC TCT GGC TCT CCC AGT AGT ACT TAT Ser Leu Arg Leu Ser Cys Asn Val Ser Gly Ser Pro Ser Ser Thr Tyr | 96 |
| | 20 25 30 | |
| 10 | TGC CTG GGC TGG TTC CGC CAG GCT CCA GGG AAG GAG CGT GAG GGG GTC Cys Leu Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val | 144 |
| | 35 40 45 | |
| 15 | ACA GCG ATT AAC ACT GAT GGC AGT GTC ATA TAC GCA GCC GAC TCC GTG Thr Ala Ile Asn Thr Asp Gly Ser Val Ile Tyr Ala Ala Asp Ser Val | 192 |
| | 50 55 60 | |
| 20 | AAG GGC CGA TTC ACC ATC TCC CAA GAC ACC GCC AAG AAA ACG GTA TAT Lys Gly Arg Phe Thr Ile Ser Gln Asp Thr Ala Lys Lys Thr Val Tyr | 240 |
| | 65 70 75 80 | |
| 25 | CTC CAG ATG AAC AAC CTG CAA CCT GAG GAT ACG GCC ACC TAT TAC TGC Leu Gln Met Asn Asn Leu Gln Pro Glu Asp Thr Ala Thr Tyr Tyr Cys | 288 |
| | 85 90 95 | |
| 30 | GCG GCA AGA CTG ACG GAG ATG GGG GCT TGT GAT GCG AGA TGG GCG ACC Ala Ala Arg Leu Thr Glu Met Gly Ala Cys Asp Ala Arg Trp Ala Thr | 336 |
| | 100 105 110 | |
| 35 | TTA GCG ACA AGG ACG TTT GCG TAT AAC TAC TGG GGC CGG GGG ACC CAG Leu Ala Thr Arg Thr Phe Ala Tyr Asn Tyr Trp Gly Arg Gly Thr Gln | 384 |
| | 115 120 125 | |
| 40 | GTC ACC GTC TCC TCA CTA GCT AGT TAC CCG TAC GAC GTT CCG GAC TAC Val Thr Val Ser Ser Leu Ala Ser Tyr Pro Tyr Asp Val Pro Asp Tyr | 432 |
| | 130 135 140 | |
| 45 | GGT TCT TAATAGAATT C Gly Ser | 449 |
| | 145 | |

(2) INFORMATION FOR SEQ ID NO: 37:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 146 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

| | | |
|----|---|--|
| | Gln Val Lys Leu Leu Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly | |
| | 1 5 10 15 | |
| 55 | Ser Leu Arg Leu Ser Cys Asn Val Ser Gly Ser Pro Ser Ser Thr Tyr | |
| | 20 25 30 | |
| 60 | Cys Leu Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val | |
| | 35 40 45 | |
| | Thr Ala Ile Asn Thr Asp Gly Ser Val Ile Tyr Ala Ala Asp Ser Val | |
| | 50 55 60 | |
| 65 | Lys Gly Arg Phe Thr Ile Ser Gln Asp Thr Ala Lys Lys Thr Val Tyr | |
| | 65 70 75 80 | |

[illegible]

(2) INFORMATION FOR SEQ ID NO: 38:

- 20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- 25 (ii) MOLECULE TYPE: DNA (genomic)

- (vii) IMMEDIATE SOURCE:
(B) CLONE: See figure 6

- 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

35

AATTTAGCGG CCGCCCAGGT GAAACTGCTC GAGTAAGTGA CTAAGGTCAC CGTCTCCTCA 60
GAACAAAAC TCATCTCAGA AGAGGATCTG AATTAATGAG AATTCATCAA ACGGTGATA 119

(2) INFORMATION FOR SEQ ID NO: 39:

- 40 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 120 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- 45 (ii) MOLECULE TYPE: DNA (genomic)

- (vii) IMMEDIATE SOURCE:
(B) CLONE: See figure 6

- 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

55 AGCTTATCAC CGTTTGATGA ATTCTCATTA ATTCAGATCC TCTTCTGAGA TGAGTTTTTG 60
TTCTGAGGAG ACGGTGACCT TAGTCACTTA CTCGAGCAGT TTCACCTGGG CGGCCGCTAA 120

(2) INFORMATION FOR SEQ ID NO: 40:

- 60 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- 65 (ii) MOLECULE TYPE: protein

- (vii) IMMEDIATE SOURCE:
(B) CLONE: See figure 6
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:
- Ala Gln Val Lys Leu Leu Glu
1 5
- (2) INFORMATION FOR SEQ ID NO: 41:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vii) IMMEDIATE SOURCE:
(B) CLONE: See figure 6
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:
- Val Thr Val Ser Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn
1 5 10 15
- (2) INFORMATION FOR SEQ ID NO: 42:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vii) IMMEDIATE SOURCE:
(B) CLONE: See figure 19
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:
- AATTTAGTCG CGACAGGTGA AACTGCTCGA GTAAGTGACT AAGGTCACCG TCTCCTCAGA 60
ACAAAACTC ATCTCAGAAG AGGATCTGAA TTAATGAGAA TTCATCTTAA GGTGATA 117
- (2) INFORMATION FOR SEQ ID NO: 43:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vii) IMMEDIATE SOURCE:
(B) CLONE: See figure 19
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:
- AGCTTATCAC CTTAAGATGA ATTCTCATTA ATTCAGATCC TCTTCTGAGA TGAGTTTTTG 60
TTCTGAGGAG ACGGTGACCT TAGTCACTTA CTCGAGCAGT TTCACCTGTC GCGACTA 117

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: See figure 19

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

Arg Gln Val Lys Leu Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: See figure 19

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

Val Thr Val Ser Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

Gln Val Lys Leu
1

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

Val Thr Val Ser Ser
1 5

- 5 (2) INFORMATION FOR SEQ ID NO: 48:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
10 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:
GTCACCGTCT CCTCATAATG A 21
- 20 (2) INFORMATION FOR SEQ ID NO: 49:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
25 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:
AGCTTCATTA TGAGGAGACG 20
- 35 (2) INFORMATION FOR SEQ ID NO: 50:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
40 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:
GTCACCGTCT CCTCATAATG ATCTTAAGGT GATA 34
- 50 (2) INFORMATION FOR SEQ ID NO: 51:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
55 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- 60 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:
AGCTTATCAC CTTAAGATCA TTATGAGGAG ACG 33
- 65

(2) INFORMATION FOR SEQ ID NO: 52:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

AATTGCGGCC GC 12

15 (2) INFORMATION FOR SEQ ID NO: 53:

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

CATGCAGTCT TCGGGC 16

30 (2) INFORMATION FOR SEQ ID NO: 54:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

TTAAGCCCGA AGACTG 16

45 (2) INFORMATION FOR SEQ ID NO: 55:

50 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

TCACTGAATT CGGGATCATG AGGACTCTCC TTGTGAGCTC GCTT 44

60 (2) INFORMATION FOR SEQ ID NO: 56:

65 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

5 ATGTCACAAA GCTTAAGCAC GAAGACAGTC GACCGTGCGG CCGGAGAC 48

(2) INFORMATION FOR SEQ ID NO: 57:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
15 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

20 CGCGTCCATG CAGTCCTCAG GTGGATCATC CCAGGTGAAA CTGC 44

(2) INFORMATION FOR SEQ ID NO: 58:

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
30 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

35 TCGAGCAGTT TCACCTGGGA TGATCCACCT GAGGACTGCA TGGA 44

(2) INFORMATION FOR SEQ ID NO: 59:

40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
45 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

50 Ser Met Gln Ser Ser Gly Gly Ser Ser Gln Val Lys Leu Leu Glu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 60:

55 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
60 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

65 CATGGCCAGG TGAAACTGCT CGAGTAAGTG ACTAAGGTCA CCGTCTCCTC AGC 53

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

GGCCCGCTGAG GAGACGGTGA CCTTAGTCAC TTACTCGAGC AGTTTCACCT GGC

53

15

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

Ser Ser Gly Gly Ser Ser
1 5

30

_

CLAIMS

1. A process for the production of an antibody or a fragment or functionalized
fragment thereof using a transformed lower eukaryotic host containing an
5 expressible DNA sequence encoding the antibody or (functionalized) fragment
thereof, wherein the antibody or (functionalized) fragment thereof is derived from
a heavy chain immunoglobulin of *Camelidae* and is devoid of light chains, and
wherein the lower eukaryotic host is a mould or a yeast.
- 10 2. A process according to claim 1, in which the mould belongs to the genera
Aspergillus or *Trichoderma*.
3. A process according to claim 1, in which the yeast belongs to the genera
Saccharomyces, *Kluyveromyces*, *Hansenula*, or *Pichia*.
- 15 4. A process according to claim 1, in which the heavy chain fragment at least
contains the whole variable domain.
5. A process according to claim 1, in which the antibody or (functionalized)
20 fragment thereof derived from a heavy chain immunoglobulin of *Camelidae*
comprises a complementary determining region (CDR) different from the CDR
belonging to the natural antibody ex *Camelidae* grafted on the framework of the
variable domain of the heavy chain immunoglobulin ex *Camelidae*.
- 25 6. A process according to claim 1, in which the immunoglobulin to be produced is a
catalytic antibody raised in *Camelidae*.
7. A process according to claim 1, in which the functionalized antibody or fragment
thereof comprises a fusion protein of both a heavy chain immunoglobulin from
30 *Camelidae* or a fragment thereof and another polypeptide.

8. A process according to claim 1, in which the DNA sequence encodes a modified heavy chain immunoglobulin or (functionalized) fragment thereof derived from *Camelidae* and being devoid of light chains, and is made by random or directed mutagenesis or both.

5

9. A process according to claim 8, in which the resulting immunoglobulin or (functionalized) fragment thereof is modified such that

- it is better adapted for production by the host cell, or
- it is optimized for secretion by the lower eukaryotic host into the
- 10 fermentation medium, or
- its binding properties (k_{on} and k_{off}) are optimized, or
- its catalytic activity is improved, or
- it has acquired a metal chelating activity, or
- its physical stability is improved.

15

10. A composition containing a product produced by a process as claimed in any one of claims 1-9.

11. New product obtainable by a process as claimed in any one of claims 1-9.

20

12. A composition containing a new product as claimed in claim 11.

* * * * *

1/20

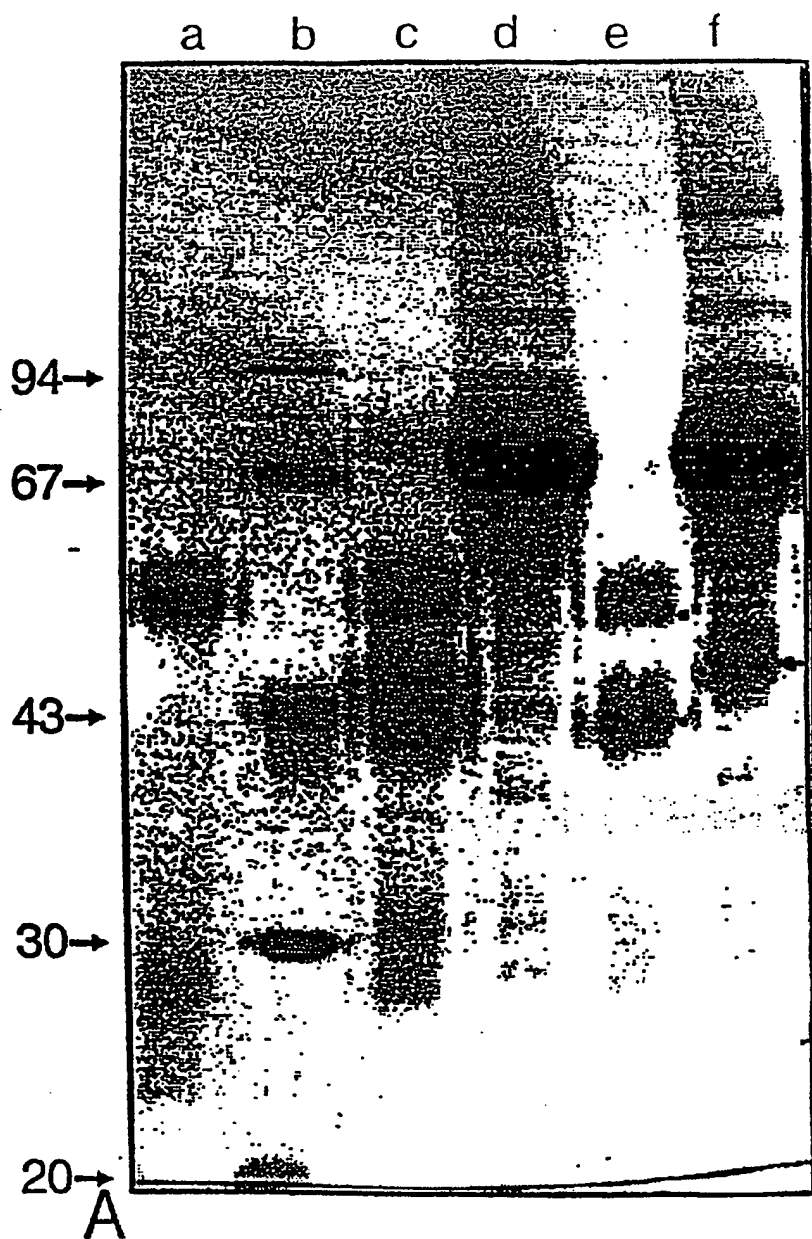


FIGURE 1A

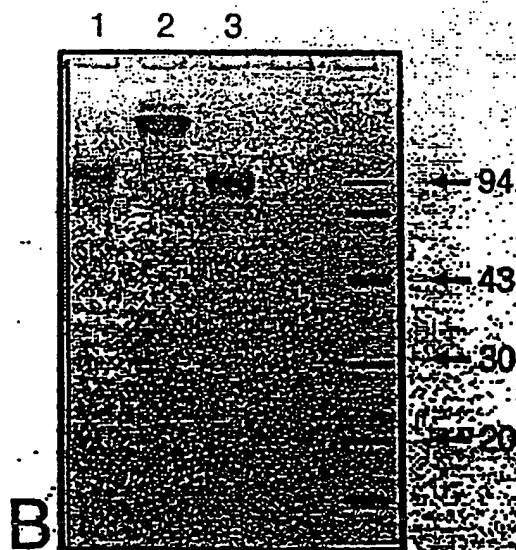


FIGURE 1B

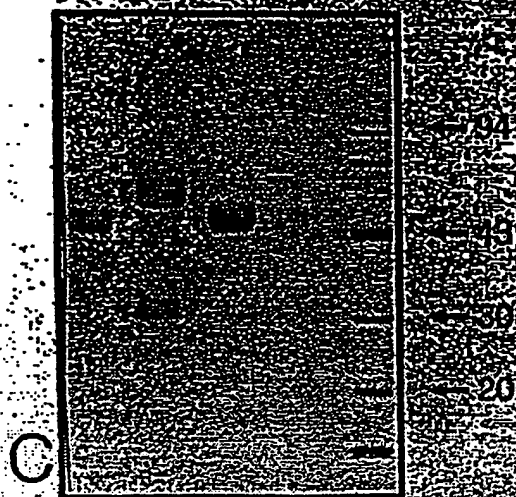
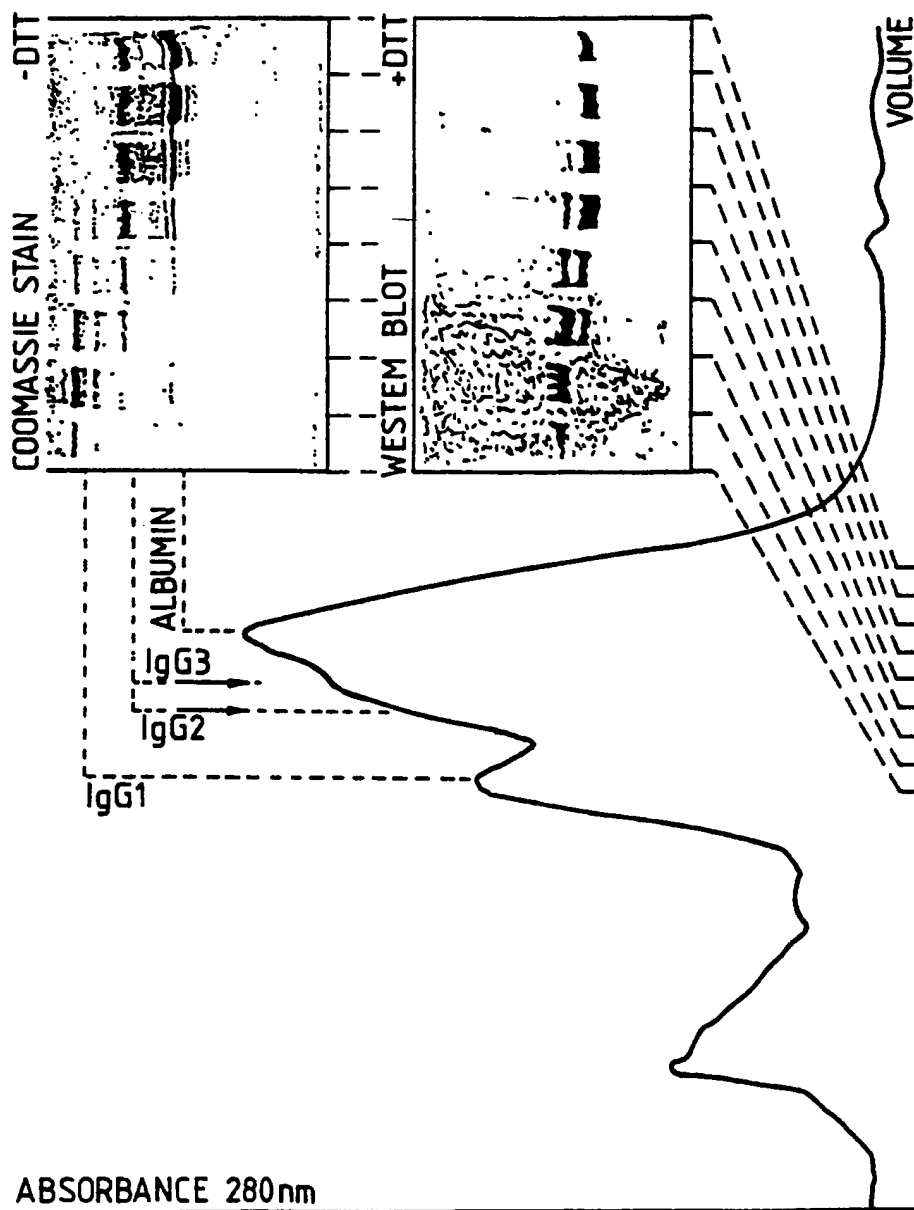


FIGURE 1C

3/20

Fig.1D



4/20

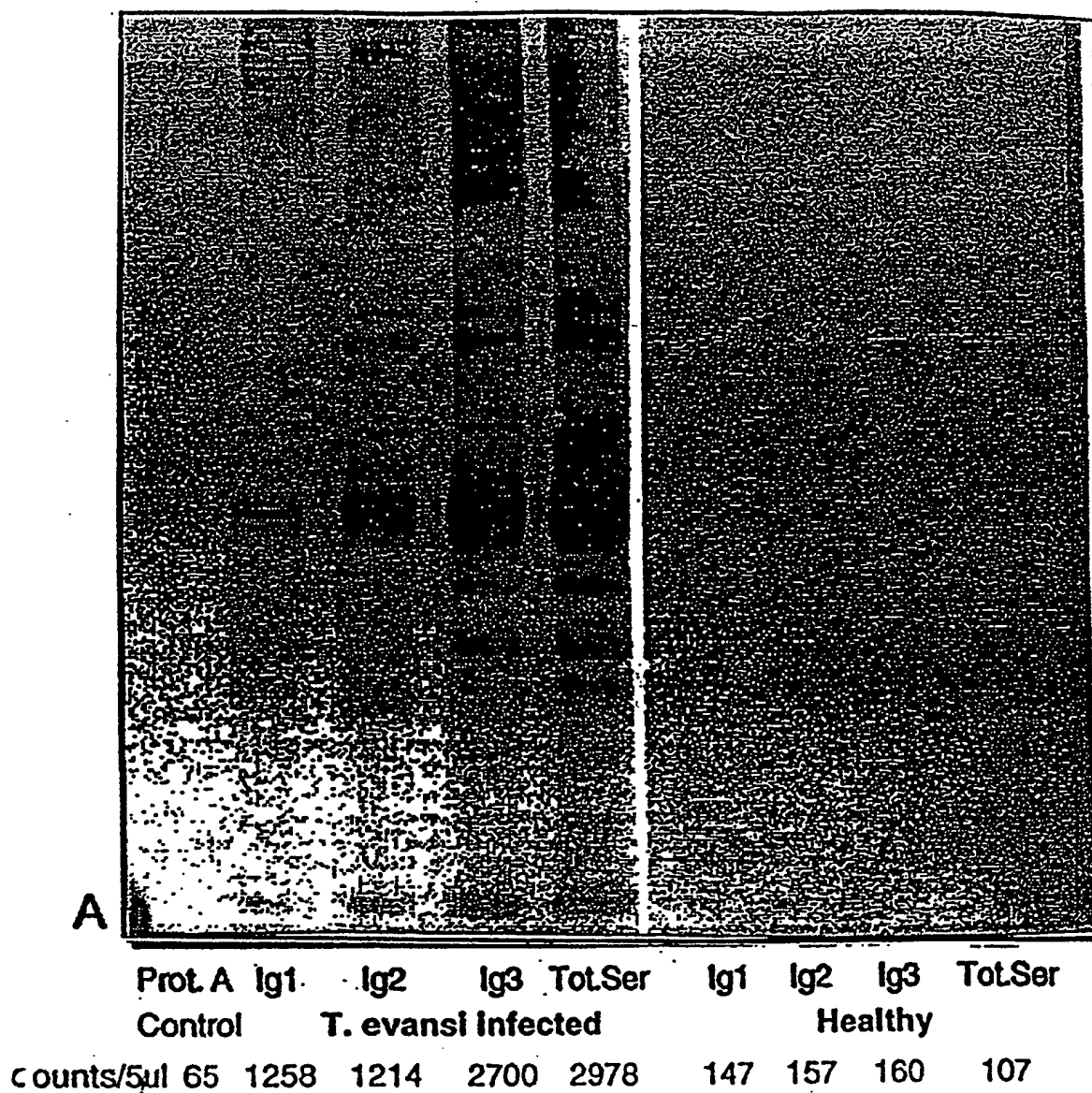


FIGURE 2A

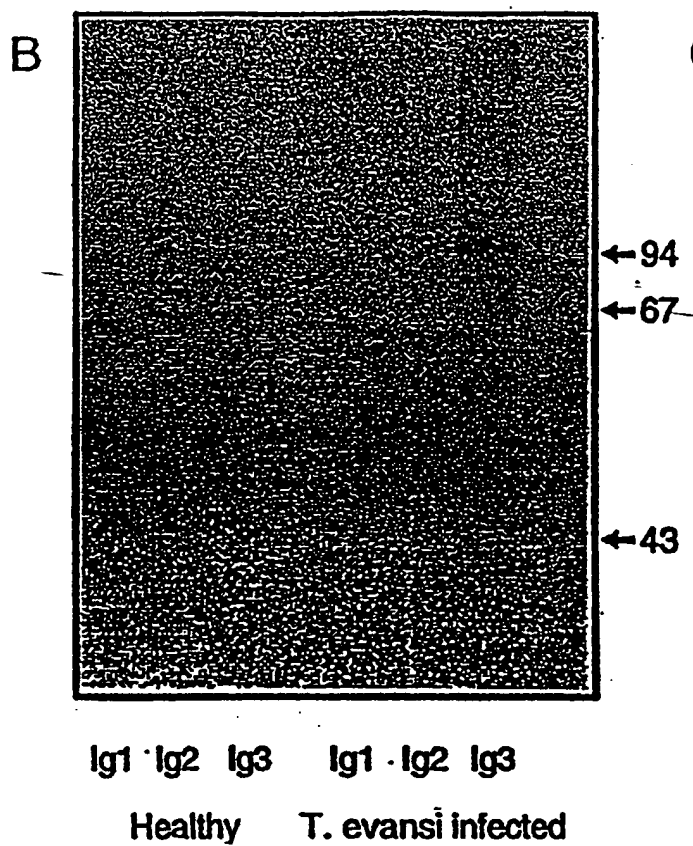


FIGURE 2B



FIGURE 2C

6/20

Fig.3.

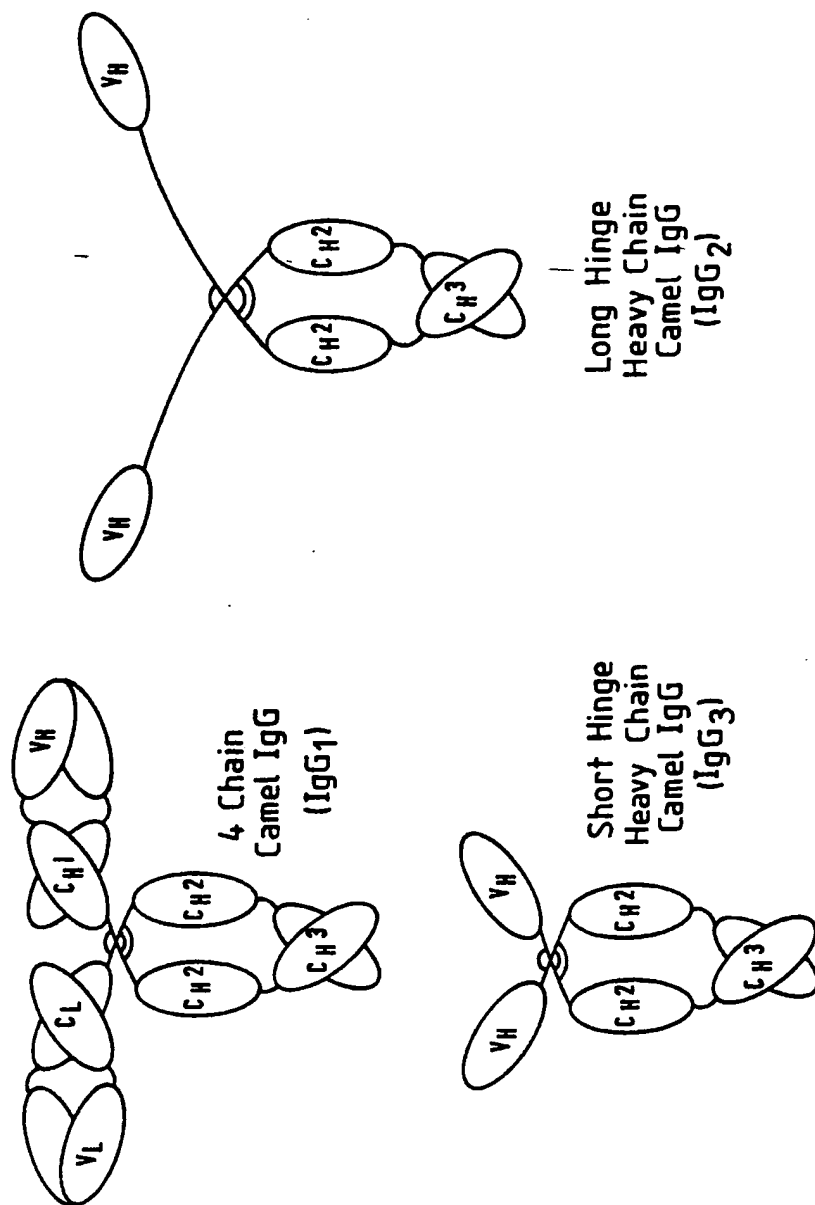
| | | | | | |
|------------|------------|--------|------|-------|-----------------|
| | 10 | 20 | | 40 | |
| EVQLVESGGG | LVQPGGSLRL | SCAASG | CDR1 | WVRQA | PGKGLEWVS CDR2 |
| GG | SVQGGGSLRL | SCAISG | CDR1 | WFREG | PGKEREGLIA CDR2 |
| GG | SVQAGGSLRL | SCASSS | CDR1 | WYRQA | PGKEREFVS CDR2 |

| | | | | |
|-------|------------|---------------|-----------|--------------|
| 70 | 80 | 90 | | 110 |
| RFTIS | RDNSKNTLYL | QMNSLRAEDTAVY | YCAR CDR3 | WGQGTQVT VSS |
| RFTIS | QDSTLKTMYL | LMNNLKPEDTGTY | YCAA CDR3 | WGQGTQVT VSS |
| RFTIS | QDSAKNTVYL | QMNSLKPEDTAMY | YCKI CDR3 | WGQGTQVT VSS |

| | | | |
|---------------|------------------------|-------------------|------------------|
| | camel V _H | hinge | C _H 2 |
| camel | WGQGTQVT VSS | GTNEVCKCPKCP | APELPGG PSVFVFP |
| | WGQGTQVT VSS | EPKIPQPQPKPQPPQ | |
| | | QPQPKPQ | |
| | | KPEPECTCPKCP | APELLGG PSVFIFP |
| ----- | | | |
| | human C _H 1 | hinge | C _H 2 |
| human gamma 3 | KVDKRV | ELKTPLGDTTHTCPRCP | |
| | | EPKCDTPPPCPRCP | |
| | | EPKSCDTPPPCPRCP | APELLGG PSVFLFP |
| human gamma 1 | KVDKK | AEPKSCDKTHTCPCP | APELLGG PSVFLFP |
| human gamma 2 | KVKVTV | ERKCCVECPCP | APPVAG - PSVFLFP |
| human gamma 4 | KVDKRV | ESKYGPPCPCP | APEFLGG PSVFLFP |

7/20

Fig.4.



8/20

Fig.5A.

XhoI

1 CAGGTGAAACTGCTCGAGTCTGGAGGAGGCTCGGTGCAGACTGGAGGATCTCTGAGACTC 60
 -----+-----+-----+-----+-----+-----+-----+-----+
 GTCCACTTTGACGAGCTCAGACCTCCTCCGAGCCACGTCTGACCTCCTAGAGACTCTGAG
 Q V K L L E S G G G S V Q T G G S L R L -

61 TCCTGTGCAGTCTCTGGATTCTCCTTTAGTACCAGTTGTATGGCCTGGTTCCGCCAGGCT 120
 -----+-----+-----+-----+-----+-----+-----+-----+
 AGGACACGT'CAGAGACCTAAGAGGAAATCATGGTCAACATACCGGACEAAGGCGGTCCGA
 S C A V S G F S F S T S C M A W F R Q A -

121 TCAGGAAAGCAGCGTGAGGGGGTCCGAGCCATTAATAGTGGCGGTGGTAGGACATACTAC 180
 -----+-----+-----+-----+-----+-----+-----+-----+
 AGTCCTTTTCGTCGCACTCCCCAGCGTCGGTAATTATCACCGCCACCATCCTGTATGATG
 S G K Q R E G V A A I N S G G G R T Y Y -

181 AACACATATGTCGCCGAGTCCGTGAAGGGCCGATTCCGCATCTCCCAAGACAAAGCCAAG 240
 -----+-----+-----+-----+-----+-----+-----+-----+
 TTGTGTATACAGCGGCTCAGGCACCTTCCGGCTAAGCGGTAGAGGGTTCTGTTGCGGTTTC
 N T Y V A E S V K G R F A I S Q D N A K -

241 ACCACGGTATATCTTGATATGAACAACCTAACCCCTGAAGACACGGCTACGTATTACTGT 300
 -----+-----+-----+-----+-----+-----+-----+-----+
 TGGTGCCATATAGAACTATACTTGTTGGATTGGGGACTTCTGTGCCGATGCATAATGACA
 T T V Y L D M N N L T P E D T A T Y Y C -

301 GCGGCGGTCCCAGCCCACTTGGGACCTGGCGCCATTCTTGATTTGAAAAAGTATAAGTAC 360
 -----+-----+-----+-----+-----+-----+-----+-----+
 CGCGGCCAGGGTGGGTGAACCCCTGGACCGCGGTAAGAACTAAACTTTTTCATATTCTATG
 A A V P A H L G P G A I L D L K K Y K Y -

BstEII

361 TGGGGCCAGGGGACCCAGGTCAACGTCTCCTCACTAGCTAGTTACCGTACGACGTTCCG 420
 -----+-----+-----+-----+-----+-----+-----+-----+
 ACCCGGTCCCGTGGGTCCAGTGGCAGAGGAGTGATCGATCAATGGGCATGCTGCAAGGC
 W G Q G T Q V T V S S L A S Y P Y D V P -

EcoRI

421 GACTACGGTTCTTAATAGAATTC 443
 -----+-----+-----+-----+-----+-----+-----+-----+
 CTGATGCCAAGAATTATCTTAAG
 D Y G S * *

9/20

Fig.5B.

XhoI

1 CAGGTGAAACTGCTCGAGTCTGGGGGAGGCTCGGTGCAGGCTGGGGGGTCTCTGACACTC 60
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 GTCCACTTTGACGAGCTCAGACCCCTCCGAGCCACGTCCGACCCCCCAGAGACTGTGAG

Q V K L L E S G G G S V Q A G G S L T L -

StyI
NcoI

61 TCTTGTGTATACACCAACGATACTGGGACCATGGGATGGTTTCGCCAGGCTCCAGGGAAA 120
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 AGAACACATATGTGGTTGCTATGACCC'TGGT'ACCC'ACC'AAAGCGGTCCGAGGT'CCCT'T'

S C V Y T N D T G T M G W F R Q A P G K -

GAGTGCGAAAGGGTCGCGCATATTACGCCTGATGGTATGACCTTCATTGATGAACCCGTG

121 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 180
 CTCACGCTTTCCCGAGCGCGTATAATGCGGACTACCATACTGGAAGTAACTACTTGGGCAC

E C E R V A H I T P D G M T F I D E P V -

AAGGGGCGATTACAGATCTCCCGAGACAACGCCAGAAAACGTTGTCTTTGCGAATGAAT

181 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 240
 TTCCCCGCTAAGTGCTAGAGGGCTCTGTTGCGGGTCTTTTGCAACAGAAACGCTTACTTA

K G R F T I S R D N A Q K T L S L R M N -

EagI

241 AGTCTGAGGCCTGAGGACACGGCCGTGTATTACTGTGCGGCAGATTGGAATACTGGACT 300
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 TCAGACTCCGGACTCCTGTGCGGGCACATAATGACACGCCGTCTAACCTTTATGACCTGA

S L R P E D T A V Y Y C A A D W K Y W T -

BstEII

301 TGTGGTGCCCGAGACTGGAGGATACTTCGGACAGTGGGGTCAGGGGGCCCGAGTCCCGTC 360
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 ACACCACGGGTCTGACCTCCTATGAAGCCTGTACCCCGAGTCCCGGGTCCAGTGGCAG

C G A Q T G G Y F G Q W G Q G A Q V T V -

EcoRI

361 TCCTCACTAGCTAGTTACCCGTACGACGTTCCGGACTACGGTTCTTAATAGAATTC 416
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 AGGAGTGATCGATCAATGGGCATGCTGCAAGGCCTGATGCCAAGAATTATCTTAAG

S S L A S Y P Y D V P D Y G S * *

10/20

Fig.5C.

XhoI

1 CAGGTGAAACTGCTCGAGTCTGGGGGAGGGTCCGGTGCAGGCTGGAGGGTCTCTGAGACTC 60
 -----+-----+-----+-----+-----+
 GTCCACTTTGACGAGCTCAGACCCCTCCAGCCACGTCCGACCTCCAGAGACTCTGAG
 Q V K L L E S G G G S V Q A G G S L R L -

61 TCCTGTAATGTCTCTGGCTCTCCCAGTAGTACTTATTGCCTGGGCTGGTTCCGCCAGGCT 120
 -----+-----+-----+-----+-----+
 AGGACATTACAGAGACCCAGAGGCTCATCATGAATAACGGACCCGACCAAGGCGGTCCCA
 S C N V S G S P S S T Y C L G W F R Q A -

121 CCAGGGAAGGAGCGTGAGGGGGTCACAGCGATTAACTGATGGCAGTGTATATACGCA 180
 -----+-----+-----+-----+-----+
 GGTCCCTTCCTCGCACTCCCCAGTGTGCTAATTGTGACTACCGTCACAGTATATGCGT
 P G K E R E G V T A I N T D G S V I Y A -

181 GCCGACTCCGTGAAGGGCCGATTACCATCTCCCAAGACACCGCCAAGAAACGGTATAT 240
 -----+-----+-----+-----+-----+
 CGGCTGAGGCACTTCCCGGCTAAGTGGTAGAGGGTCTGTGGCGGTTCTTTTGCCATATA
 A D S V K G R F T I S Q D T A K K T V Y -

241 CTCCAGATGAACAACCTGCAACCTGAGGATACGGCCACCTATTACTGCGCGGCAAGACTG 300
 -----+-----+-----+-----+-----+
 GAGGTCTACTTGTGGACGTTGGACTCCTATGCCGGTGGATAATGACGCGCCGTTCTGAC
 L Q M N N L Q P E D T A T Y Y C A A R L -

301 ACGGAGATGGGGGCTTGTGATGCGAGATGGGCGACCTTAGCGACAAGGACGTTTTCGTAT 360
 -----+-----+-----+-----+-----+
 TGCCTCTACCCCOGAACACTACGCTCTACCGCTGGAATGCTGTTCTGCAAACGCATA
 T E M G A C D A R W A T L A T R T F A Y -

BstEII

361 AACTACTGGGGCCGGGGGACCCAGGTACCGTCTCCTCACTAGCTAGTTACCGGTACGAC 420
 -----+-----+-----+-----+-----+
 TTGATGACCCCGGCCCCCTGGGTCCAGTGGCAGAGGAGTGATCGATCAATGGGCATGCTG
 N Y W G R G T Q V T V S S L A S Y P Y D -

EcoRI

421 GTTCCGGACTACGGTTCTTAATAGAATTC 449
 -----+-----+-----+-----+
 CAAGGCCTGATGCCAAGAATTATCTTAAG
 V P D Y G S * *

Fig. 6.

(EcoRI) EagI XhoI BstEII
 AATTTAGCGGCCCGCCAGGTGAAACTGCTCGAGTAAAGTGACTAAGGTCACCGTCTCCTCA + 60
 1 AATCGCGGGGGTCCACTTTGACGAGCTCATTCAGTCCAGTGGCAGAGGAGT V T V S S
 A Q V K L L E
 61 GAACAAAACTCATCTCAGAAGAGGATCTGAATTAATGAGAATTCATCAACGGTGATA HindIII
 CTTGTTTTGAGTAGAGTCTTCTCCTAGACTTNAATTACTCTTAAGTAGTGTGGCCACTATT + 120
 E Q K L I S E E D L N *
 121 --- 123
 CGA

Fig. 19.

(EcoRI) NruI XhoI BstEII
AATTTAGTCGGACAGGTGAAACTGCTCGAGTAAAGTCAAGGTCAACGGTCTCCTCAGA
+-----+-----+-----+-----+-----+-----+-----+-----+
1 ATCAGCGCTGTCCACTTTGACGAGCTCATTCACCTGATTCGAGTGGCAGAGGAGTCT 60
R Q V K L L V T V S S E

EcoRI AflII HindIII
ACAAAACATCATCTCAGAGAGGATCTGAATTAATGAGAAATTCATCTTAAGGTGATA
+-----+-----+-----+-----+-----+-----+-----+-----+
61 TGTTTTGGAGTAGAGTCTTCTCTAGACTTAATTACTCTTAAGTAGAATTCCACTATTGG 120
Q K L I S E E D L N * *

121 - 121
A

12/20

Fig.7.

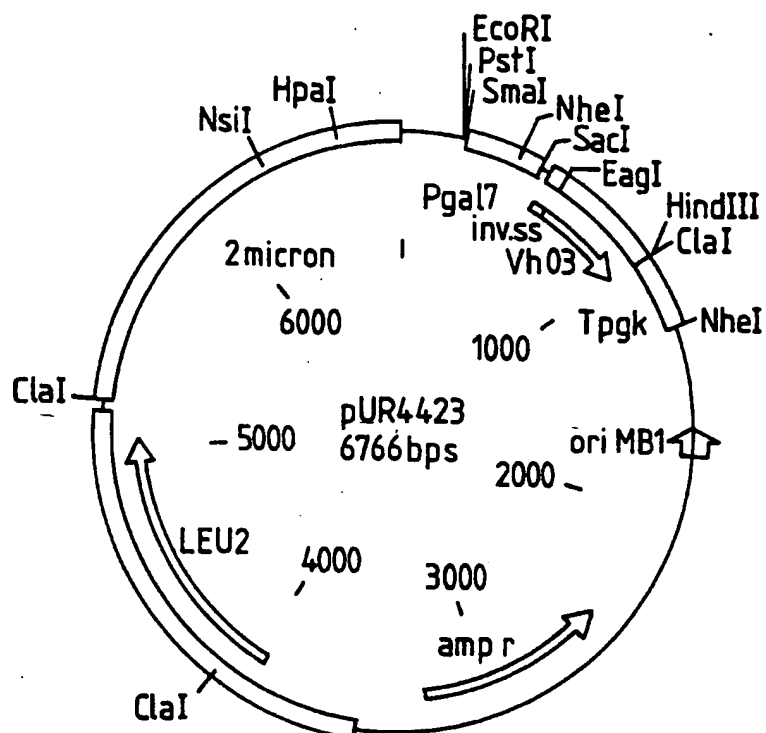
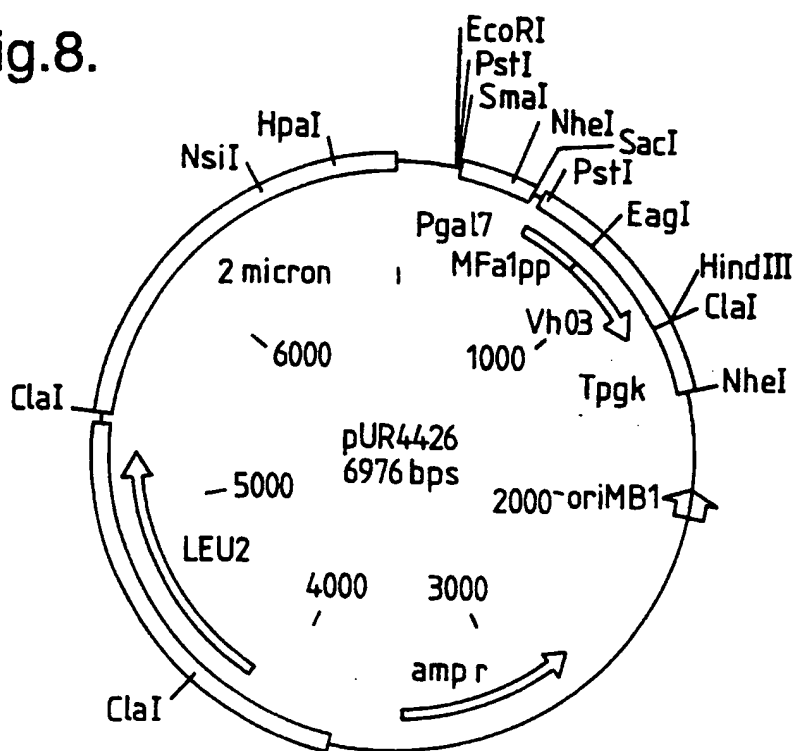


Fig.8.



13/20

Fig.9.

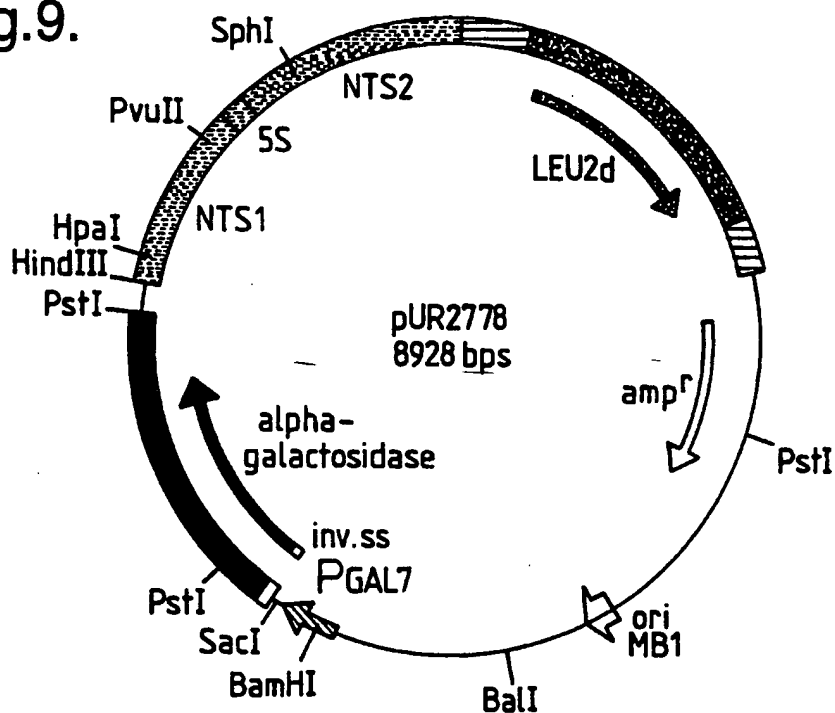
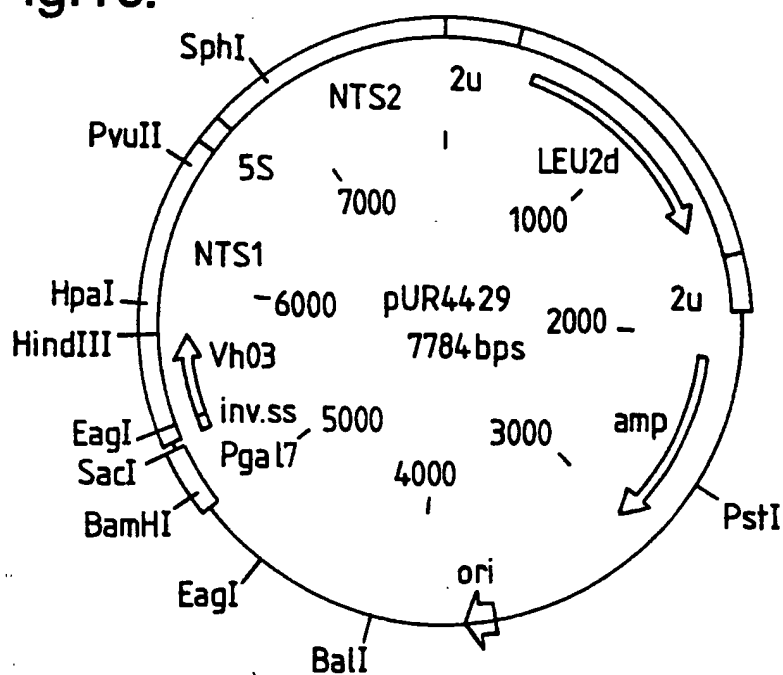


Fig.10.



14/20

Fig.11.

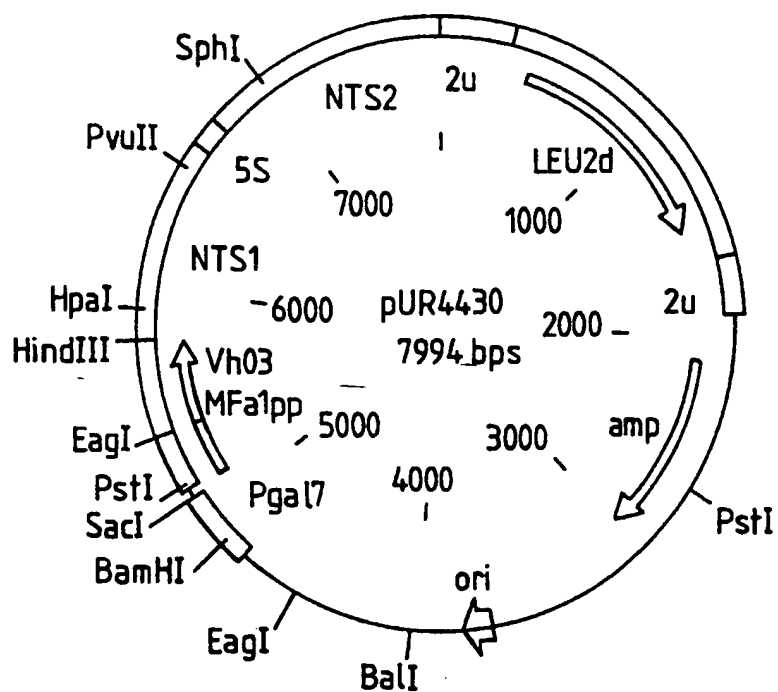
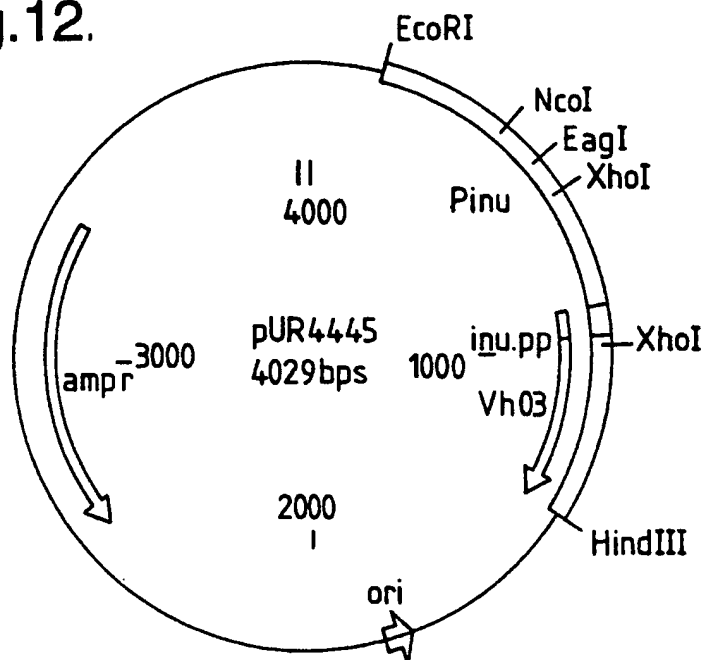


Fig.12.



15/20

Fig.13.

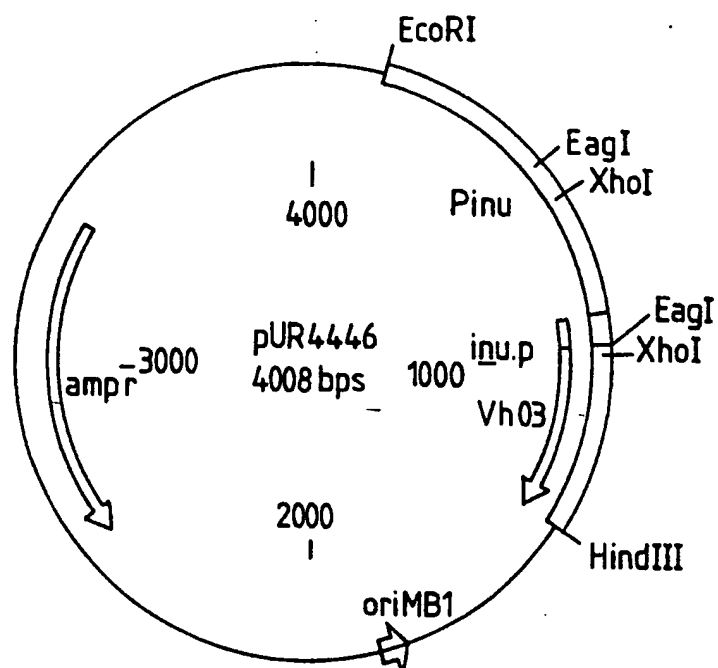
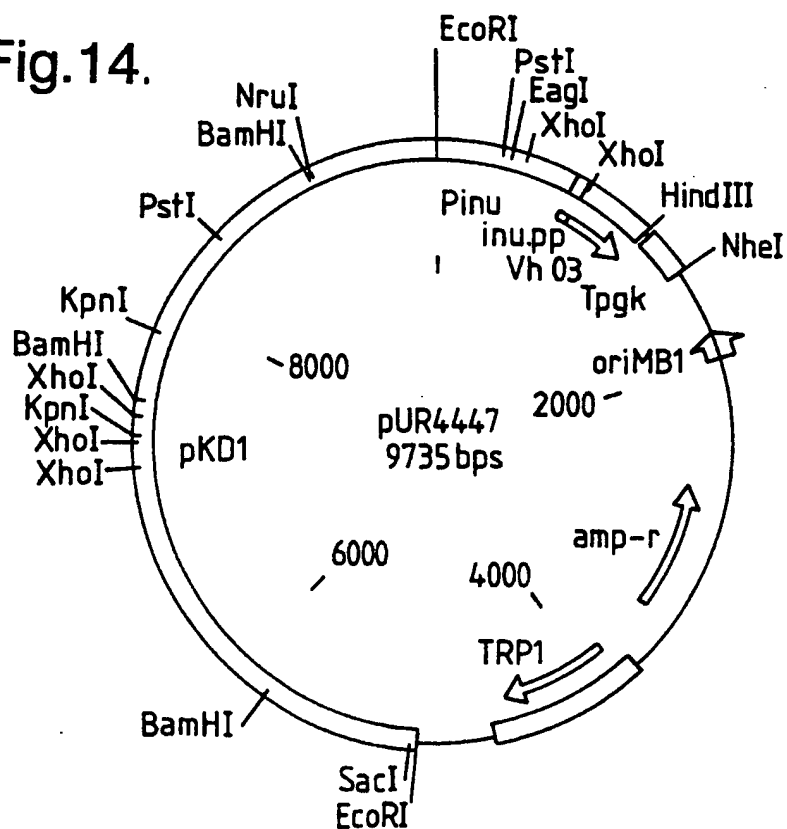


Fig.14.



16/20

Fig.15.

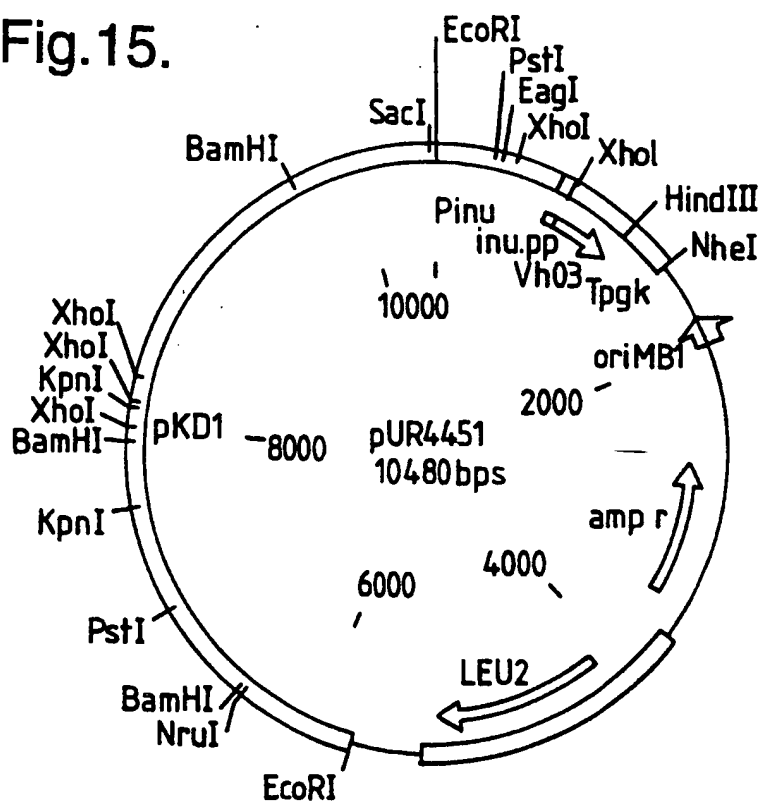
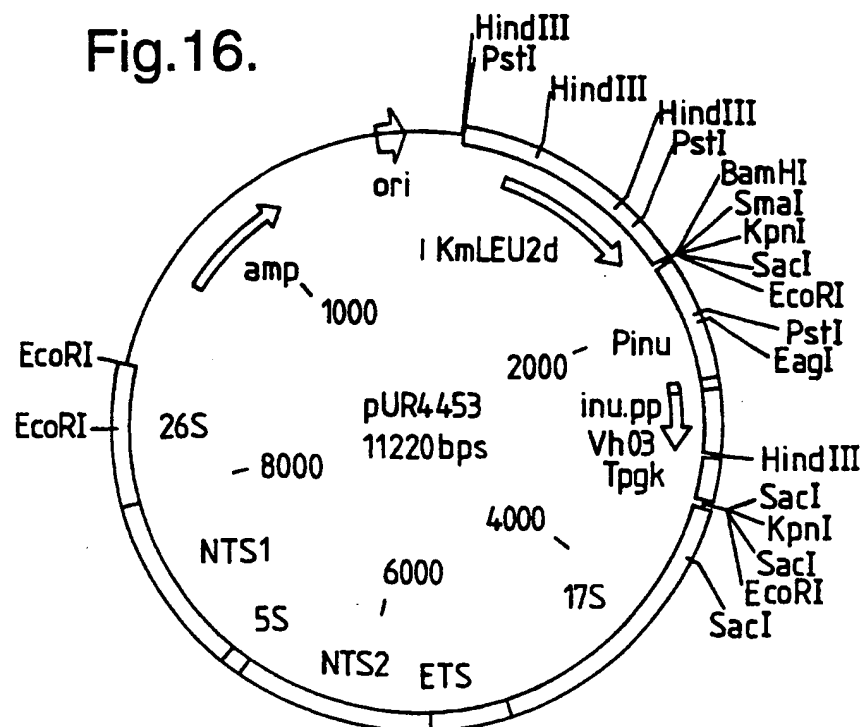
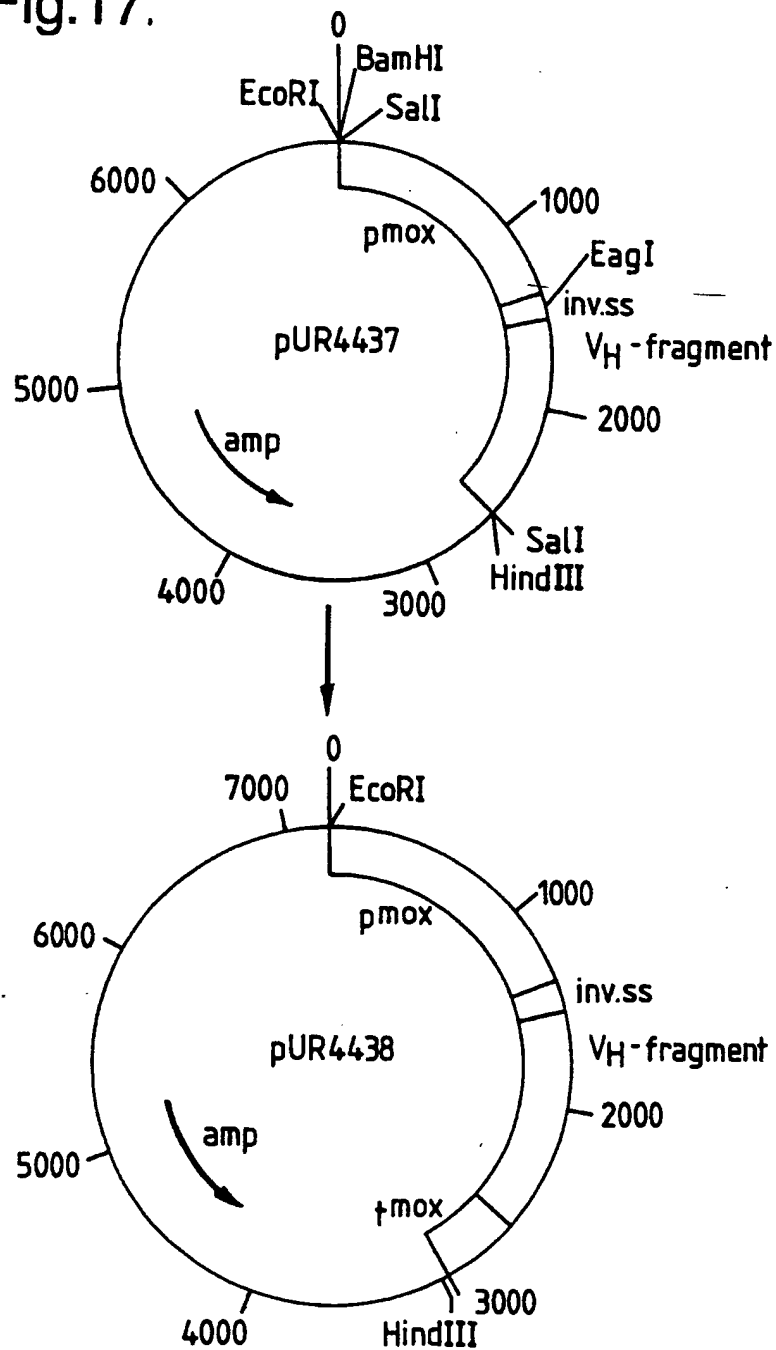


Fig.16.



17/20

Fig.17.



18/20

Fig.18.

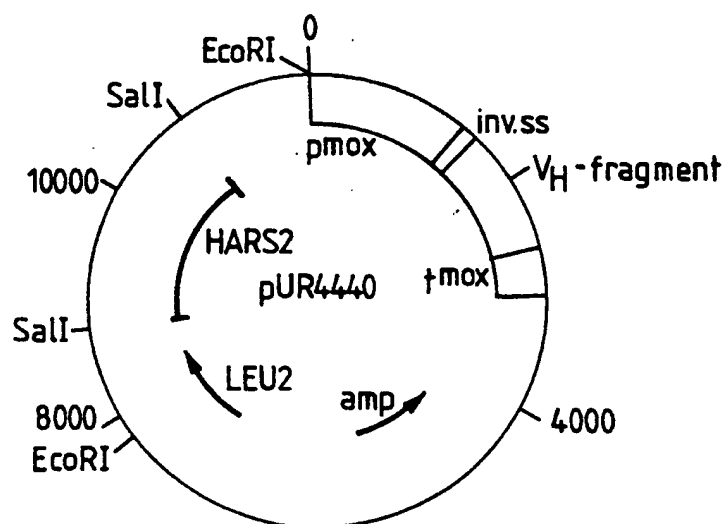
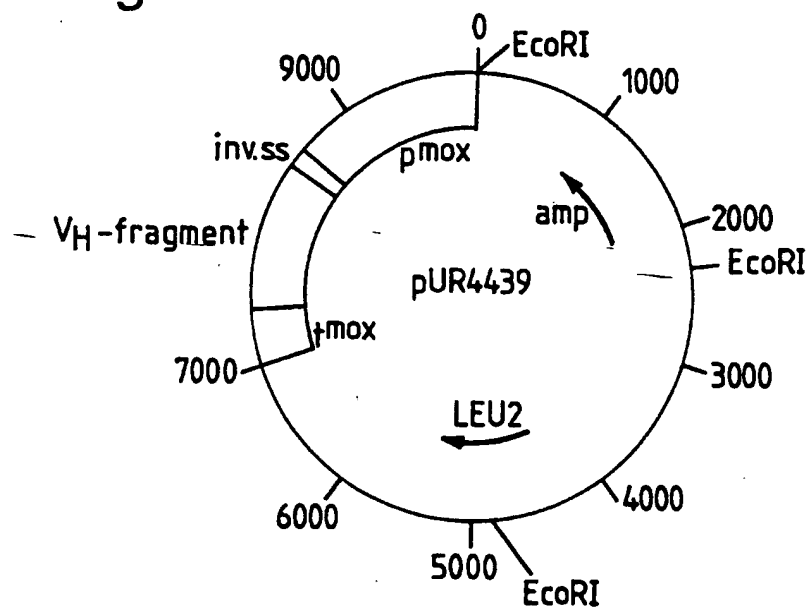
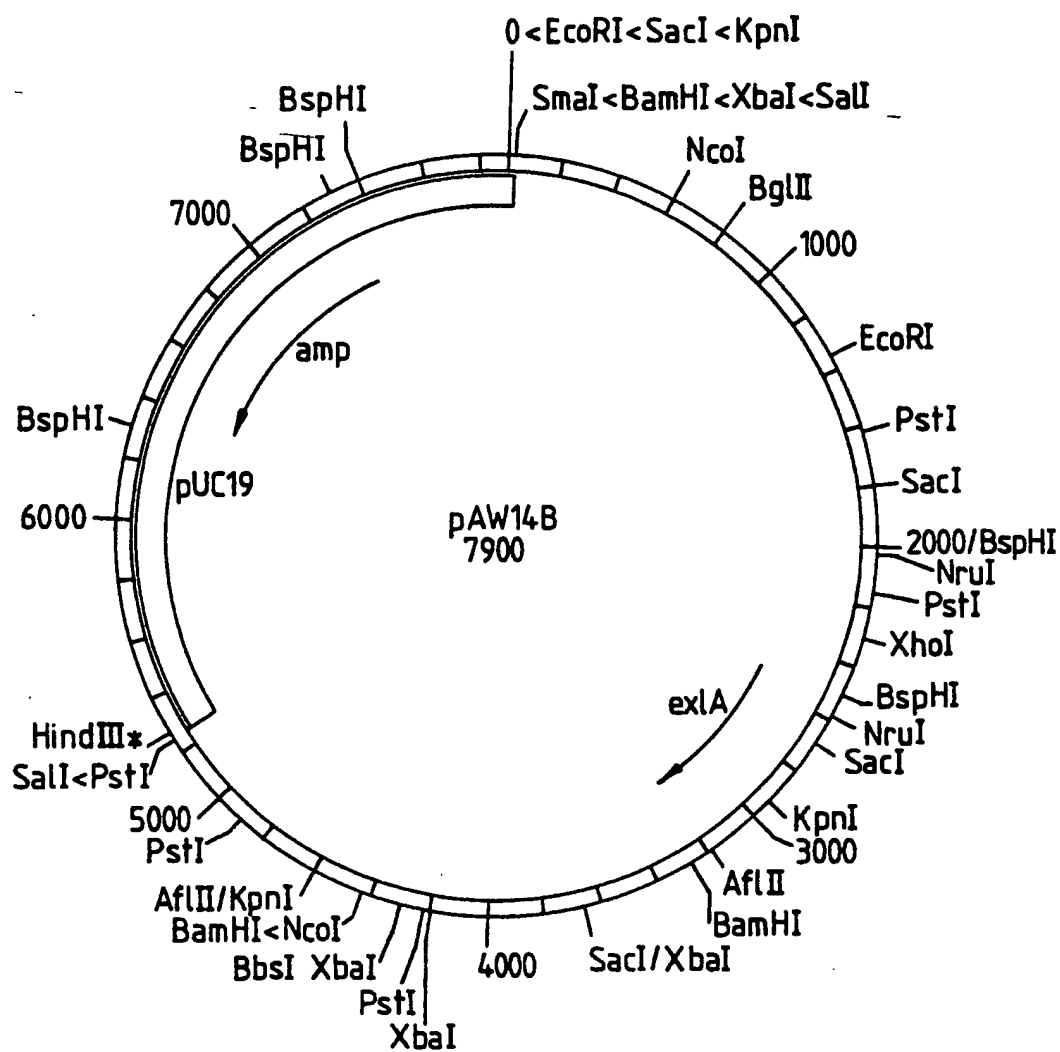


Fig.20.



20/20

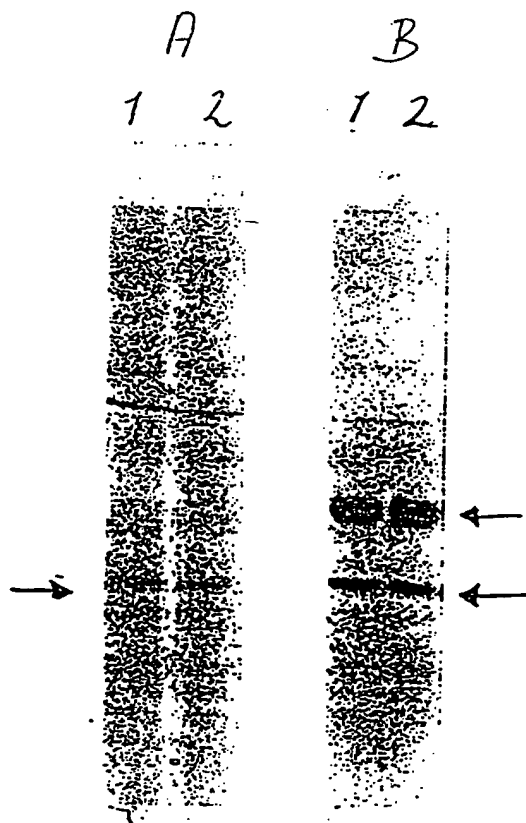


FIGURE 21

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 94/01442

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/13 C07K15/28 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|---|-----------------------|
| A | EP,A,0 256 421 (PHILLIPS PETROLEUM COMPANY) 24 February 1988 cited in the application see the whole document --- | 1,3 |
| P,X | NATURE vol. 363, no. 6428, 3 June 1993, LONDON, GB pages 446 - 448 C. HAMERS-CASTERMAN ET AL. 'Naturally occurring antibodies devoid of light chains.' cited in the application see the whole document --- -/-- | 1,4, 10-12 |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

A document member of the same patent family

Date of the actual completion of the international search

19 August 1994

Date of mailing of the international search report

26-08-1994

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Nooij, F

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 94/01442

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| P,X | FEBS LETTERS vol. 339, no. 3 , 21 February 1994 , AMSTERDAM, THE NETHERLANDS pages 285 - 290 J. DAVIES ET AL. 'Camelising' human antibody fragments: NMR studies on VH domains.' see the whole document --- | 1,5, 10-12 |
| P,X | WO,A,94 04678 (C. CASTERMAN ET AL.) 3 March 1994 see the whole document ----- | 1,3,4,6, 10-12 |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 94/01442

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
| EP-A-0256421 | 24-02-88 | AU-B- 620667 | 20-02-92 |
| | | AU-A- 4590789 | 22-03-90 |
| | | AU-B- 594476 | 08-03-90 |
| | | AU-A- 7474787 | 18-02-88 |
| | | JP-A- 63044899 | 25-02-88 |
| <hr/> | | | |
| WO-A-9404678 | 03-03-94 | EP-A- 0584421 | 02-03-94 |
| | | AU-B- 4949793 | 15-03-94 |
| <hr/> | | | |

1. The first part of the document is a list of the names of the members of the committee.